A FIELD INVESTIGATION OF MARINE ANEMIA IN FARMED SALMON IN BRITISH COLUMBIA

A Thesis Submitted to the College of Graduate Studies and Research in Partial Fulfilment of the Requirements for the Degree of Doctor of Philosophy in the Department of Veterinary Microbiology University of Saskatchewan Saskatoon

By Robert Craig Stephen

Spring, 1995

© Copyright R. Craig Stephen, 1995. All rights reserved.
The author has granted a non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of this thesis in microform, paper or electronic formats.

The author retains ownership of the copyright in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

L’auteur a accordé une licence non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de cette thèse sous la forme de microfiche/film, de reproduction sur papier ou sur format électronique.

L’auteur conserve la propriété du droit d’auteur qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

0-612-23929-2
UNIVERSITY OF SASKATCHEWAN

College of Graduate Studies and Research

SUMMARY OF DISSERTATION

Submitted in partial fulfillment
of the requirements for the

DEGREE OF DOCTOR OF PHILOSOPHY

by

R. Craig Stephen

Department of Veterinary Microbiology
University of Saskatchewan

Spring 1994

Examinining Committee:

Dr. P. Flood

Dean/Associate Dean/Dean’s Designate
College of Graduate Studies and Research

Dr. L. Polley

Chair of Advisory Committee, Department
of Veterinary Microbiology

Dr. C. Ribble

Supervisor, Department of Herd Medicine
and Theriogenology

Dr. M. Kent

Pacific Biological Station, Department
of Fisheries and Oceans

Dr. G. Wobeser

Department of Veterinary Pathology

Dr. J. Ellis

Department of Veterinary Microbiology

Dr. J. Campbell

Department of Herd Medicine and
Theriogenology

External Examiner:

Dr. D. Waltner-Toews
Department of Population Medicine
University of Guelph
Guelph, Ontario
A Field Investigation of Marine Anemia in Farmed Salmon in British Columbia

This investigation was designed to describe the medical ecology of marine anemia as it occurred in salmon populations in British Columbia in order to develop and evaluate causal and intervention hypotheses.

The historical case definition of marine anemia was evaluated by conducting an observer variation trial. A new working case definition was developed by reviewing features of previously diagnosed cases and applying mathematical decision rules. Retrospective and prospective observational methods were used to describe the changing spatial, temporal, and host distributions of the disease. Behavioural observations of marked moribund fish and mortality surveys were employed to evaluate methods of sampling for marine anemia in seapens. Finally, a descriptive epidemiological study was conducted to describe the impact of marine anemia in British Columbia, to identify potential causal factors, and to suggest possible avenues for control.

Marine anemia was described as an endemic disease of farmed chinook salmon in British Columbia. The broad demographic characteristics of the disease and its limited impact suggested that the introduction of a new pathogen was probably not the "cause" of the emergence of marine anemia. Historical problems with identifying the disease in individuals and populations suggested that marine anemia is more likely a newly described disease than a truly new disease. The reliance on surface moribund fish as indicators of the prevalence and impact of
marine anemia in seafarms potentially biased previous
descriptions of the disease. When an explicit case definition,
applicable to all members of the seapen population was used, the
disease could not be associated with excessive mortality rates.
The diagnosis was, however, associated with a variety of factors
capable of stimulating an excessive immune response.

Future control efforts should be directed towards decreasing
the overall level of infectious disease in seapens, as well as
social and physiological stressors inherent in current techniques
of seapen farming. Our results emphasized the importance of
studying a disease in a population and ecological context before
intervention and causal inferences can be accepted.
PERMISSION TO USE STATEMENT

In presenting this thesis in partial fulfilment of the requirements for a degree of Doctor of Philosophy from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying this thesis in any manner, in whole or in part, for scholarly purposes may be granted by the professor who supervised my thesis work or, in his absence, by the Head of the Department of Veterinary Microbiology or the Dean of the Western College of Veterinary Medicine. It is understood that any copying or publication or use of this thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis.

Requests for permission to copy or to make other use of material in this thesis in whole or part should be addressed to:

Head, Department of Veterinary Microbiology.
Western College of Veterinary Medicine.
University of Saskatchewan, Saskatoon. S7N 0W0.
ABSTRACT

A FIELD INVESTIGATION OF MARINE ANEMIA IN FARmed SALMON IN
BRITISH COLUMBIA

R. Craig Stephen  SuperviSor:
University of Saskatchewan, 1994  Dr. Carl S. Ribble

This investigation was designed to describe the medical ecology of marine anemia as it occurred in salmon populations in British Columbia in order to develop and evaluate causal and intervention hypotheses.

The historical case definition of marine anemia was evaluated by conducting an observer variation trial. A new working case definition was developed by reviewing features of previously diagnosed cases and applying mathematical decision rules. Retrospective and prospective observational methods were used to describe the changing spatial, temporal, and host distributions of the disease. Behavioural observations of marked moribund fish and mortality surveys were employed to evaluate methods of sampling for marine anemia in seapens. Finally, a descriptive epidemiological study was conducted to describe the impact of marine anemia in British Columbia, to identify potential causal factors, and to suggest possible
avenues for control.

Marine anemia was described as an endemic disease of farmed chinook salmon in British Columbia. The broad demographic characteristics of the disease and its limited impact suggested that the introduction of a new pathogen was probably not the "cause" of the emergence of marine anemia. Historical problems with identifying the disease in individuals and populations suggested that marine anemia is more likely a newly described disease than a truly new disease. The reliance on surface moribund fish as indicators of the prevalence and impact of marine anemia in sea farms potentially biased previous descriptions of the disease. When an explicit case definition, applicable to all members of the seapen population was used, the disease could not be associated with excessive mortality rates. The diagnosis was, however, associated with a variety of factors capable of stimulating an excessive immune response.

Future control efforts should be directed towards decreasing the overall level of infectious disease in seapens, as well as social and physiological stressors inherent in current techniques of seapen farming. Our results emphasized the importance of studying a disease in a population and ecological context before intervention and causal inferences can be accepted.
ACKNOWLEDGEMENTS

Funding for this research was provided by the Science Council of British Columbia, the British Columbia Ministry of Agriculture, Fisheries and Food, the Wildlife Health Fund and the Deans MRC Fund of the Western College of Veterinary Medicine, and Moore-Clark Company of Canada. Gracious support in kind was provided by the Pacific Biological Station (Department of Fisheries and Oceans), and many owners and managers of salmon farms throughout southern British Columbia.

My supervising professor, Dr. Carl Ribble, has had a tremendous impact on the way I think about epidemiology. Though his guiding hand was often subtle, I can now see how well Carl steered me through my degree. I am proud to call Carl my teacher, colleague, and friend.

I am indebted to Dr. Lydden Polley who provided me the freedom to mature as a scientist, and to Dr. Michael Kent whose critical ear and willingness to be challenged were, to me, the signs of a true scientist. Finally, I thank Dr. John Iverson. Our conversations always remind me that thinking about disease is fun.
For Sharon, Paul and David

Your patience and understanding made it possible
TABLE OF CONTENTS

Permission to use i
Abstract iv
Acknowledgements vi
Table of Contents
List of Tables
List of Figures
List of Appendices

1. INTRODUCTION AND LITERATURE REVIEW

   Introduction to the disease 1
   Investigative approach 6
   References 17

2. THE EFFECTS OF CHANGING DEMOGRAPHICS ON THE
   DISTRIBUTION OF MARINE ANEMIA IN FARMED SALMON IN
   BRITISH COLUMBIA

   Introduction 27
   Materials and Methods 29
      Sources of disease information 29
      Demographic data 33
      Case finding 33
      Analysis 35
   Results 36
   Discussion 46
   References 52

3. AN EVALUATION OF SURFACE MORIBUND SALMON AS
   INDICATORS OF SEAPEN DISEASE STATUS

   Introduction 56
   Materials and Methods 57
      Setting and design of mark-recapture trials 57
      Pathological evaluation 59
      Behavioural observations 63
   Results 64
   Discussion 72
   References 79
4. OBSERVER VARIATION IN THE HISTOLOGIC DIAGNOSIS OF PLASMACYTOID LEUKEMIA (MARINE ANEMIA)

<table>
<thead>
<tr>
<th>Section</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>85</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>87</td>
</tr>
<tr>
<td>Selection of samples and participants</td>
<td>87</td>
</tr>
<tr>
<td>Diagnostic categories</td>
<td>89</td>
</tr>
<tr>
<td>Analysis</td>
<td>89</td>
</tr>
<tr>
<td>Results</td>
<td>90</td>
</tr>
<tr>
<td>Discussion</td>
<td>96</td>
</tr>
<tr>
<td>References</td>
<td>105</td>
</tr>
</tbody>
</table>

5. MARINE ANEMIA IN FARmed CHINOOK SALMON (ONCORYHNCHUS TSHAWYTSCHA): DEVELOPMENT OF A WORKING CASE DEFINITION

<table>
<thead>
<tr>
<th>Section</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>111</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>113</td>
</tr>
<tr>
<td>Sources of data</td>
<td>113</td>
</tr>
<tr>
<td>Development and evaluation of histologic criteria</td>
<td>114</td>
</tr>
<tr>
<td>Development and evaluation of gross criteria</td>
<td>116</td>
</tr>
<tr>
<td>Results</td>
<td>118</td>
</tr>
<tr>
<td>Discussion</td>
<td>128</td>
</tr>
<tr>
<td>References</td>
<td>133</td>
</tr>
</tbody>
</table>

6. MORTALITY SURVEYS AS A TOOL FOR STUDYING MARINE ANEMIA IN SEAPEN REARED SALMON

<table>
<thead>
<tr>
<th>Section</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>138</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>141</td>
</tr>
<tr>
<td>Sources of data</td>
<td>141</td>
</tr>
<tr>
<td>Diagnostic criteria</td>
<td>142</td>
</tr>
<tr>
<td>Analysis</td>
<td>144</td>
</tr>
<tr>
<td>Results</td>
<td>145</td>
</tr>
<tr>
<td>Discussion</td>
<td>152</td>
</tr>
<tr>
<td>References</td>
<td>156</td>
</tr>
</tbody>
</table>
7. DESCRIPTIVE EPIDEMIOLOGY OF MARINE ANEMIA IN SEAPEN REARED CHINOOK SALMON IN SOUTHERN BRITISH COLUMBIA

Introduction 160
Materials and Methods 162
   Mail survey 163
   Case finding 164
   Mortality survey 167
Results 169
Discussion 181
References 190

8. CONCLUSIONS 201

References 219
# LIST OF TABLES

1.1 Fundamental steps of an outbreak investigation 8

1.2 Characteristics of 45 previously published investigations of diseases of farmed fin-fish 12

1.3 A list of previously published reports of disease of farmed fin-fish reviewed for this report 13

2.1 The changing demographic distribution of reported cases of marine anemia in British Columbia from 1987 to 1992 37

2.2 Proportion of total chinook submissions by region and by year according to veterinary clinical records from 1987-1992 41

2.3 Marine anemia status of all diagnostic submissions according to veterinary records for 1987-1992 43

2.4 Highest possible true prevalence of marine anemia for regional samples detecting 0% marine anemia (based on 95% confidence levels for estimates of prevalence as in Sackett *et al.* 1993, p.176) 45

3.1 Examples of diseases commonly diagnosed in chinook seapens in British Columbia (Kent, 1992; Hicks, 1989, Brackett, 1991) classified according to the diagnostic categories developed for this study 62

3.2 Post-tagging survival time of moribund salmon captured at the surface of seapens 65

3.3 Proportional mortality of marked and unmarked moribund seapen reared salmon 68

3.4 Distribution of body condition scores of moribund fish recovered in tagging trials 69

4.1 Observed and beyond chance agreement of the histologic classification of plasmacytoid leukemia 91

4.2 *Kappa* values for the mean inter- and intra-pathologist diagnosis of plasmacytoid leukemia 93
4.3 Mean inter-pathologist agreement on the histological diagnosis of plasmacytoid leukemia for comparisons of pathologists B to C, C to D, and D to B 95

5.1 Distribution of lesions in specific organs in positive cases of marine anemia 119

5.2 Frequency and significance of gross pathological lesions associated with marine anemia 124

5.3 Group-level sensitivity (GSENS) and specificity (GSPEC) for different levels of marine anemia prevalence (MA PREV) and number of salmon sampled per pen. Criteria for declaring a pen positive = one positive fish per group 127

6.1 Farm-to-farm variation in the number of surface moribund (SCF) and dead fish accessible for examination 146

6.2 Factors associated with the proportion of dead fish in seapens unsuitable for marine anemia diagnosis based on linear regression analysis 150

6.3 Correlation matrix of factors associated with the proportion of dead fish unsuitable for marine anemia diagnosis in seapens based on Pearson’s correlation coefficient 151

7.1 Sources of marine anemia cases in farmed and wild Pacific salmon 173

7.2 Mean prevalence of diagnostic categories observed during mortality surveys on chinook (Oncorhynchus tshawytscha) seafarms 179
LIST OF FIGURES

2.1 Major salmon farming regions in southern British Columbia 30

2.2 Annual number of British Columbia chinook salmon (Oncorhynchus tshawytscha) farm licences issued per region 39

2.3 Proportion of laboratory and clinical submissions classified as positive for marine anemia 42

5.1 Mean tubule score and proportion of large cells in the renal interstitium of apparently healthy fish and fish with marine anemia 121

5.2 Algorithm for the diagnosis of marine anemia in chinook salmon using gross pathological signs 126

7.1 General locations of capture of wild and farmed salmon for the descriptive study of marine anemia 168

7.2 Seasonal distribution of the proportion of submissions to veterinarians and diagnostic laboratories diagnosed as marine anemia (1987-1992). 176

7.2 Seasonal distribution of the number of cases of marine anemia found on commercial salmon farms 177
LIST OF APPENDICES

3.1 Morbidity and mortality characteristics of farms participating in the tagging trials 84

4.1 Proportional intra and inter-pathologist agreement for the diagnosis of positive (PL+), negative (PL-), and questionable (PL?) cases of plasmacytoid leukemia 110

6.1 Relationship of post-mortem gill colour and quality of tissues for gross and histopathologic examination 159

7.1 Marine anemia mail survey sent to randomly selected licensed chinook salmon seafarms in British Columbia 196

7.2 Morbidity and mortality information from adjacent "matched" seafarms 200
CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Introduction to the disease

The harvest of fish from the coastal waters off British Columbia (B.C.), has long been a source of food and income for many Canadians. Recently, aquaculture has played an increasing role in providing marketable fish products. However, one of the major limiting factors for the growth and development of the B.C. salmon farming industry is disease (Anon, 1992). In 1992, estimates of the direct losses from disease ranged from 15-30% of fish prior to harvest, an economic loss of $20-30 million annually (Anon, 1992). In the fall of 1988 an apparently new disease began to contribute to these losses. In the five years following its discovery, the disease, commonly known as "marine anemia", became a source of economic, public health, and scientific concern. The history and nature of marine anemia in the B.C. salmon farming industry is the subject of this work.

In the autumn of 1988, several chinook salmon (Oncorhynchus tshawytscha) farms in the Sechelt Inlet of
southern B.C. began to experience epidemics of mortality. Preliminary investigations were unable to attribute these losses to any commonly known cause of seapen salmon mortality. Moribund fish collected from affected farms demonstrated an unusual combination of gross pathological lesions that included marked pallor of the gills, renosplenomegaly, ascites and, in some cases, severe exophthalmia. Although this combination of signs is shared by other common infectious diseases of seapen salmon, the lack of other significant lesions, such as granulomas, fibrin, or pustules, led investigators to suspect that the epidemics of mortality were the result of a new, distinct syndrome (Anon, 1989). Because of the marked gill pallor of affected fish, the disease was referred to as marine anemia.

Histological examination of affected fish provided the defining characteristics of marine anemia. All cases of the disease exhibited prominent proliferation of actively dividing hemoblasts in a variety of organs. Affected tissues included the renal interstitium, spleen, liver, pancreas, lamina propria of the pyloric caeca and colon, heart, skeletal muscle, and retrobulbar tissues. Although many of the fish were infected with potential pathogens, such as Renibacterium salmoninarum or Enterocytozoan salmonis, a number of cases were found where hemoblast proliferation was the only significant
post mortem finding (Kent et al, 1990).

The ultrastructural characteristics of the proliferating cells were consistent with those of immature piscine plasma cells (Kent et al, 1990). The detection in tissue section of immunoglobulin in these cells supported the description of the hemoblasts as plasma cell precursors (Kent et al, 1990). The immaturity of the cells, and their aggressive invasion and proliferation in a wide variety of tissues suggested that the disease was neoplastic (Kent et al, 1990). Based on these findings, marine anemia was described as a "plasmacytoid leukemia".

Initial estimates of the impact of the disease ranged from 50-80% mortality in market-sized fish (Kent et al, 1990; Newbound and Kent, 1991). As marine anemia was discovered on more farms in the Sechelt Inlet, the range of the magnitude of losses attributed to the disease widened. At some farms, the impact was negligible, while others claimed nearly 100% mortality (Kent, 1993). Some farmers chose to prematurely market fish after marine anemia had been diagnosed at their sites in order to avoid anticipated heavy losses (Kent, 1993). Because the disease was initially diagnosed primarily in high input, market-sized fish, industry representatives became concerned about the potential for devastating economic losses on affected farms. A number of the more severely affected sites identified losses from marine anemia as the primary
reason for the financial failure of their farms (Brackett et al., 1990). Because chinook farms predominated in the B.C. salmon farming industry in the 1980's, aquaculturists became concerned that the effects of marine anemia seen on individual farms could multiply throughout the entire industry. When marine anemia was subsequently diagnosed in important salmon-rearing regions other than the Sechelt Inlet, salmon farming representatives listed the investigation of marine anemia as an industry priority (Brackett et al., 1990; Anon, 1992).

Based on early observations of the disease, and the association of leukemias in other species with infectious agents, initial research efforts concentrated on finding a causative pathogen and on determining its route of transmission (Newbound and Kent, 1991; Anon, 1993; Kent and Dawe, 1993). The lesions of marine anemia were soon replicated in laboratory studies by the intraperitoneal injection of crude homogenates of renal tissue from affected salmon into recipient fish (Kent and Dawe, 1990). Although more difficult to achieve, the disease was also replicated when recipient fish were injected intraperitoneally with cell free filtrates that had been passed through 0.22 micron filters (Kent and Dawe, 1990; Kent and Dawe, 1993). Transmission by ultra-filtered tissue homogenates suggested that larger pathogens, such as bacteria or protozoa, were not responsible for the
disease (Kent and Dawe, 1993). Therefore, research efforts focused on identifying a viral cause of marine anemia (Eaton and Kent, 1992; Kent and Dawe, 1993).

The discovery of particles of retroviral morphology in tissues of naturally and experimentally produced cases of marine anemia encouraged investigators to seek additional evidence to establish a retroviral etiology for the disease (Eaton and Kent, 1992). Reverse transcriptase assays and purified viral polypeptide analyses were conducted on natural and experimental cases of the disease. The results of these studies, coupled with electron microscopic observations, lead to the description of a new retrovirus, the salmon leukemia virus (Eaton and Kent, 1992). This virus was proposed as the probable agent involved in the etiology of marine anemia (Eaton and Kent, 1992).

The potential for epidemics of infectious cancer in Pacific salmon populations raised the interest of scientists and the public. In December 1991, marine anemia became the subject of mass media attention. Concerns over public health and food safety, coupled with a perceived threat to wild salmon stocks, emphasized the need to understand the nature of marine anemia and to develop management strategies for the disease. Despite the growing public and industry concern regarding marine anemia, few studies undertook the task of systematically assessing the
nature and magnitude of the problems created by the disease as it occurred on commercial salmon farms.

**Investigative approach**

A new disease can be studied in two settings, artificial and natural (Susser, 1973). By providing careful descriptions of pathophysiological mechanisms, laboratory studies conducted in carefully controlled settings can provide insights into causal mechanisms at the level of the individual animal (Susser, 1973; Hanson, 1988). Although an understanding of causality at this level is important, it alone will not allow one to resolve a disease problem (Hancock and Wikse, 1988). As the concepts of multiple causality and multi-factorial diseases have developed, anomalies and defects in laboratory-based "theories of causality" have became apparent. (Schwabe, 1993). Human tuberculosis is an example of this realization. While it is certain that *Mycobacterium tuberculosis* fulfills all of Koch's postulates for tuberculosis in individuals, this is not the same as saying the tubercle bacillus causes outbreaks of the disease in a population. The most effective method of decreasing the incidence and impact of tuberculosis has come from improving the socio-economic environment of the susceptible population and not by directly attacking the agent with pharmaceutical agents (Lewontin, 1991). The
inability of laboratory studies to replicate the complex ecological mechanisms required to create and maintain a naturally occurring disease outbreak limits the ability of such research to accurately characterize an outbreak, as well as minimizing the chances of providing effective control recommendations (Hanson, 1988).

Virchow said "The study of things caused, must precede the study of the cause of things" (Elliott and Tattersfield, 1979). The objective of a field investigation is to carefully study a disease and accurately characterize the problems created by the disease under natural conditions. Unlike the "classic scientific method", the goal of a field investigation of a disease problem is not to confirm hypotheses by experimentation (Meek, 1993). Instead, by systematically studying a disease in an ecological context, a field investigator attempts to determine the key factors that control the occurrence and behaviour of a disease in nature (Hancock and Wikse, 1988). These observations then act as the basis for the creation of causal and intervention hypotheses (Schwabe et al., 1977).

There are four principal components of a field-based disease investigation (Table 1.1). Each component contributes to the ultimate objective of identifying factors that can be manipulated to reduce the incidence and impact of a disease (Hancock and Wikse, 1988).
Table 1.1. Fundamental steps of an outbreak investigation

1. Define and verify the problem.
   - establish and validate a reliable case definition
   - quantify and verify the impact of the disease on the
     individual and the group

2. Describe the medical ecology of the problem
   - orient the problem as to its spatial, temporal,
     environmental, and host pattern.

3. Describe and quantify risk factors.
   - compare and contrast groups with different levels of
     disease

4. Develop hypotheses for the intervention plan
Relating the disease status of individuals and populations to risk factors is a prerequisite for population based studies of disease (Susser, 1973). Accordingly, establishing a reliable means of diagnosing and detecting the disease of concern should be the first step in an outbreak investigation. By providing consistent rules for determining the presence or absence of a disease, a reliable case definition increases the likelihood of detecting important disease associations (Holmes et al., 1988). In addition, the representative selection and complete detection of cases within a population are essential for unbiased descriptions of the extent of a disease in a population (Martin et al., 1987, p.262). Once the disease can be readily diagnosed and detected, the magnitude of the problems created by the disease can be measured and verified.

As most outbreaks of disease are a result of an imbalance in the ecological relationship of disease determinants within a host population (Martin et al., 1987, p.253), an essential component of a field investigation is to describe the medical ecology of the disease of concern. Ecology, derived from the Greek word oikos, meaning house or habitation, is concerned with factors that affect the distribution and abundance of biological entities. Medical ecology is, therefore, the study of "where a disease lives". It is the branch of science that describes and
measures factors that influence, or are influenced by, the distribution and maintenance of disease in populations. Spatial, temporal, environmental, and host patterns often provide important clues as to the factors that create and sustain a disease (Schwabe et al., 1977). Understanding the relative importance of these factors is essential for the rational development of disease assessment and control programs (Martin et al., 1987, p.253).

The comparison of the level of disease in different populations is a fundamental epidemiological method used to associate causal or preventative factors with a disease (Martin et al., 1987, p.129-132). A third component of field investigations is, therefore, to describe and quantify the factors that put individuals and populations at risk for developing a disease. Unbiased comparisons of the level of disease in populations under different ecological conditions help to identify and prioritize factors that may be exploited to control a disease.

Platt (1964) suggested that the difference between a productive and a non-productive hypothesis is the quality of thought and observation that act as the basis for generating the hypothesis. By defining and quantifying the occurrence and behaviour of a disease, observations derived from field investigations form a basis for developing hypotheses about the cause and control of a disease. The validity of the hypotheses generated by a
field investigation, therefore, depends on the quality of its observations. Measurement of associations between a disease and causal factors can be biased in an observational study whenever uncontrolled or unseen factors systematically affect the results or conclusions of the study (Sackett, 1979; Weinberg, 1993). The measurement and prevention of biases and confounding are therefore central issues in the design and analysis of field investigations. By considering the biology of the disease and the affected population when designing, conducting and analyzing outbreak investigations, spurious associations in descriptive studies can be substantially reduced (Kelsey et al., 1986).

A review of previous reports of health disorders in farmed fish revealed that the model for disease investigations described above has rarely been applied on fish farms (Table 1.2). The reports selected for review were published in refereed animal and fish health journals between 1974 and 1993 (Table 1.3). To be selected for review, the stated objective of the paper had to be that of investigating aspects of a naturally occurring disease in cultured fish. The methods employed by the authors of these reports were typical of those used to investigate disease problems in British Columbia farmed salmon. Most of the studies were conducted in response to elevated mortality rates. Although many of the reports presented
Table 1.2. Characteristics of 45 reports of disease investigations of farmed fish disease.

1. Case definition
   - 36% determined the disease status of fish by the presence or absence of a pathogen.
   - 48% defined a case by the presence of specific post-mortem lesions.
   - 15% provided clinical and pathological or microbiological criteria for diagnosis.

2. Problem definition
   - 38% provided no description of how diagnostic samples were collected, 14% used haphazard or convenience sampling strategies, 46% relied on sub-samples of selected members of the population (most often, surface caught moribund fish).
   - 28% quantified the impact of the problem by providing overall mortality rates, but none gave measures of cause-specific impact.
   - 2% compared risk factors in affected and unaffected groups.
   - 33% of the reports followed the progression of the disease over time.

3. Descriptive epidemiology
   - 48% gave minimal descriptions of demographic and environmental information for affected groups.
   - 9% described ecological features of affected and unaffected groups.

4. Causal inference
   - 70% provided explanations for the cause of the outbreak or provided intervention recommendations.
Table 1.3. Reviewed reports of disease in farmed fish.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Disease/Pathogen</th>
<th>Fish</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brockelbank et al</td>
<td>1993</td>
<td>rickettsia</td>
<td>salmon</td>
</tr>
<tr>
<td>Hatai and Hoshiai</td>
<td>1992</td>
<td>saprolegnia</td>
<td>salmon</td>
</tr>
<tr>
<td>Poppe et al</td>
<td>1992</td>
<td>hexamita</td>
<td>salmon</td>
</tr>
<tr>
<td>Palmer et al</td>
<td>1992</td>
<td>hematopoietic necrosis</td>
<td>salmon</td>
</tr>
<tr>
<td>Novoa et al</td>
<td>1992</td>
<td>various pathogens</td>
<td>turbot</td>
</tr>
<tr>
<td>Keranen et al</td>
<td>1992</td>
<td>various pathogens</td>
<td>whitefish</td>
</tr>
<tr>
<td>Kent et al</td>
<td>1992</td>
<td>hexamita</td>
<td>salmon</td>
</tr>
<tr>
<td>Foott et al</td>
<td>1992</td>
<td>E.I.B.S.</td>
<td>salmon</td>
</tr>
<tr>
<td>Groff et al</td>
<td>1992</td>
<td>hepatopathy</td>
<td>striped bass</td>
</tr>
<tr>
<td>Murphy et al</td>
<td>1992</td>
<td>pancreas disease</td>
<td>salmon</td>
</tr>
<tr>
<td>Bruno</td>
<td>1992</td>
<td>ichthyobodo</td>
<td>salmon</td>
</tr>
<tr>
<td>Rodger et al</td>
<td>1991</td>
<td>myopathy</td>
<td>salmon</td>
</tr>
<tr>
<td>Austin and Stobie</td>
<td>1991</td>
<td>unknown bacteria</td>
<td>trout</td>
</tr>
<tr>
<td>Branson et al</td>
<td>1991</td>
<td>rickettsia</td>
<td>salmon</td>
</tr>
<tr>
<td>Cvitanich et al</td>
<td>1991</td>
<td>rickettsia</td>
<td>salmon</td>
</tr>
<tr>
<td>Kent and Margolis</td>
<td>1991</td>
<td>gilquinia squali</td>
<td>salmon</td>
</tr>
<tr>
<td>Kieser et al</td>
<td>1991</td>
<td>lymphoma</td>
<td>salmon</td>
</tr>
<tr>
<td>Rodger</td>
<td>1991</td>
<td>diphyllobothrium</td>
<td>salmon</td>
</tr>
<tr>
<td>Burtle et al</td>
<td>1991</td>
<td>myxozoan</td>
<td>catfish</td>
</tr>
<tr>
<td>Hastein and Lindstad</td>
<td>1991</td>
<td>various pathogens</td>
<td>salmon</td>
</tr>
<tr>
<td>Kent et al</td>
<td>1990</td>
<td>marine anemia</td>
<td>salmon</td>
</tr>
<tr>
<td>Morrison et al</td>
<td>1990</td>
<td>lymphoblastosis</td>
<td>salmon</td>
</tr>
<tr>
<td>Hedrick et al</td>
<td>1990</td>
<td>enterocytozoan</td>
<td>salmon</td>
</tr>
<tr>
<td>Kent</td>
<td>1990</td>
<td>netpen liver disease</td>
<td>salmon</td>
</tr>
</tbody>
</table>
Table 1.3 continued

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Organism</th>
<th>Host</th>
</tr>
</thead>
<tbody>
<tr>
<td>McLean and Herman</td>
<td>1989</td>
<td>herpesvirus</td>
<td>trout</td>
</tr>
<tr>
<td>Rasheed</td>
<td>1989</td>
<td>vibrio</td>
<td>seabream</td>
</tr>
<tr>
<td>Deardorf and Kent</td>
<td>1989</td>
<td>anakasis</td>
<td>salmon</td>
</tr>
<tr>
<td>Kent * et al.</td>
<td>1989</td>
<td>loma</td>
<td>salmon</td>
</tr>
<tr>
<td>Kent * et al.</td>
<td>1988</td>
<td>paramoeba</td>
<td>salmon</td>
</tr>
<tr>
<td>Kent * et al.</td>
<td>1988</td>
<td>cytophaga</td>
<td>salmon</td>
</tr>
<tr>
<td>Kent * et al.</td>
<td>1988</td>
<td>toxic hepatic</td>
<td>salmon</td>
</tr>
<tr>
<td>pathology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferguson * et al.</td>
<td>1987</td>
<td>tetrahymena</td>
<td>salmon</td>
</tr>
<tr>
<td>Hawke * et al.</td>
<td>1987</td>
<td>pasteurellosis</td>
<td>bass</td>
</tr>
<tr>
<td>Harrell * et al.</td>
<td>1986</td>
<td>rosette agent</td>
<td>salmon</td>
</tr>
<tr>
<td>Foo * et al.</td>
<td>1985</td>
<td>streptococcus</td>
<td>signids</td>
</tr>
<tr>
<td>Roberts</td>
<td>1983</td>
<td>yersinia</td>
<td>trout</td>
</tr>
<tr>
<td>Cann and Taylor</td>
<td>1982</td>
<td>botulism</td>
<td>trout</td>
</tr>
<tr>
<td>Egidius * et al.</td>
<td>1981</td>
<td>vibrio</td>
<td>salmon</td>
</tr>
<tr>
<td>Roberts * et al.</td>
<td>1979</td>
<td>pancreatitis</td>
<td>trout</td>
</tr>
<tr>
<td>Roberts and Horne</td>
<td>1978</td>
<td>meningitis</td>
<td>trout</td>
</tr>
<tr>
<td>Blaxter * et al.</td>
<td>1974</td>
<td>vitamin-B</td>
<td>herring</td>
</tr>
<tr>
<td></td>
<td></td>
<td>deficinency</td>
<td></td>
</tr>
</tbody>
</table>

Sources of reports:
- a Canadian Veterinary Journal
- b Journal of Wildlife Disease
- c Disease of Aquatic Organisms
- d Journal of Fish Disease
- e Aquaculture
- g Journal of Aquatic Animal Health
causal explanations for the disease outbreaks, potential biases and confounding factors identified in the design of these studies threatened the validity of their conclusions. Instead of characterizing the nature of the disease problem in a population and ecological context, the majority of the reports emphasized the characterization of pathological lesions or possible pathogens. Potentially biased sampling strategies, restrictive case definitions, and the failure to compare groups were common features of a number of these reports. Causal associations were generally based on fulfilling variants of Koch’s postulates. As the majority of these papers focused on the description of the pathologic and microbiological features of severe cases selected from non-random sub-samples of the affected farms, it was generally not possible to accept causal explanations for the outbreaks described based on the evidence provided. It is important to emphasize that the ability of researchers to apply the method of disease investigation that is described in Table 1.1 is often limited by the management conditions encountered on fish farms. However, the lack of an ecological or population information, the reviewed reports of disease outbreaks on fish farms failed to provide unbiased information from which reliable intervention plans or causal hypotheses could confidently be based.
By conducting a field investigation of marine anemia in B.C. salmon farms, it was our objective to document the extent of the problems created by the disease and to provide epidemiological information that could be used for the development of sound control and prevention programs. There are two principal questions regarding marine anemia that must be addressed before intervention plans can be developed: (1) Have elements of the ecology, management, or dynamics of the B.C. chinook farming industry affected our perception of the nature, cause and impact of marine anemia?; (2) Can we reliably diagnose and detect the full spectrum of marine anemia and its effects so that associations that are discovered among the disease, its natural history, and risk factors are not biased or confounded? The following chapters each deal with aspects of these two basic questions. By studying marine anemia as it naturally exists, it was the intent of this work to document the impact and ecological aspects of the disease in order to provide new insights into the nature of marine anemia.
References


CHAPTER 2

THE EFFECTS OF CHANGING DEMOGRAPHICS ON THE DISTRIBUTION OF MARINE ANEMIA IN FARmed SALMON IN BRITISH COLUMBIA

Introduction

The changing geographic pattern of an evolving disease outbreak can reveal much about the genesis and maintenance of the disease in nature. This is particularly so in the epidemiologic investigation of neoplastic disorders. The search for spatial clusters or changes in the geographic distribution of specific cancers has provided important data for the generation and testing of causal hypotheses (Walter, 1992). Biogeographic studies of diseases are, however, faced with the challenge of separating true non-random spatial patterns of disease from spurious patterns which are, instead, only a reflection of the distribution and the method of sampling of the population at risk (Schwabe et al, 1977).

Marine anemia, also known as plasmacytoid leukemia, is a recently described disease affecting farmed chinook salmon (Oncorhynchus tshawytscha) in British Columbia (B.C.), Canada. The disease has been implicated as the
cause of massive mortalities in B.C salmon farms (Kent et al, 1990). Because of its association with a newly described retrovirus (Eaton and Kent, 1992), it has been suggested that, like retroviral leukemias of other animals, marine anemia could spread throughout the population at risk (Brackett et al, 1991). Evidence supporting the hypothesis that marine anemia is a spreading, infectious, neoplastic disease could have profound regulatory effects on the salmon farming industry.

Although B.C.’s aquaculture industry dates back to the early 1900’s, salmon farming itself is new to the province. Since its inception in the 1970’s, salmon farming has rapidly expanded to become one of the largest animal agriculture systems in B.C. (Egan and Kenney, 1993). Salmon farming experienced a period of rapid growth in B.C. from 1985 to 1992, when it entered a stage of transition and rationalization (Anon, 1992). In a search for better water quality, and fewer conflicts with upland owners and other resources, the industry expanded from its origins in the Sechelt Inlet on the mainland of B.C. to a variety of regions along the coastline of the southern mainland and Vancouver Island (Anon, 1992).

In this paper, the changing geographic patterns of marine anemia diagnoses are compared with the changing demographics of the salmon farming industry in B.C. in order to examine the hypothesis that marine anemia is a
disease that spread from its original site of detection to all of the major fish farming regions of the province.

Materials and Methods
Sources of disease information

The five major regions of farmed salmon production in B.C. are illustrated in Figure 2.1. Areas other than those shown in the figure were listed under the category "outer coast". This latter region was principally represented by the Prince Rupert area of northern B.C., but also included a small number of reports from mainland locations northeast of Vancouver Island.

The distribution of cases of marine anemia between 1987 and 1992 was established retrospectively by reviewing three sources of information. First, the clinical records of five aquaculture veterinarians were collected. From 1987 to 1992, veterinary services were principally supplied to the B.C. salmon farmers by veterinarians employed by four companies providing feed or pharmaceuticals to the industry. The records from two of these companies were not used because either there was insufficient detail in the records to allow for confident data extrapolation, or the company was limited in the number of farms and geographic areas their veterinarians visited. The area of practice for the veterinarians involved included all of the regions shown in Figure 2.1. The location, species and clinical
Figure 2.1  Major salmon farming regions in southern British Columbia. (C=Central, S=South, W=West, NW=Northwest, N=North)
diagnoses of each submission were recorded. It is important to note that a "submission" was not equivalent to a single fish but was instead the record of a single farm visit. Veterinarians usually collected clinical and post-mortem information from more than one fish during a farm visit. To be classified as a positive submission, the clinical record had to specify marine anemia as a final diagnosis, with supporting clinical and pathological evidence recorded.

The second source of data was a review of the diagnostic records of aquaculture submissions to two pathology laboratories: a federal fish health laboratory (Department of Fisheries and Oceans, Nanaimo, B.C.), and the provincial Animal Health Centre (B.C. Ministry of Agriculture, Fisheries and Food, Abbotsford, B.C.). These two laboratories received submissions from all of the major fish farming regions in the province. They were responsible for most of the diagnostic support available to the B.C. aquaculture industry during the six years covered by the retrospective portion of this study. Information identical to that collected in the review of veterinary clinical records was recorded for the laboratories. Historical information was often lacking with the laboratory submissions, so the laboratory diagnosis of marine anemia depended, in most cases, almost exclusively on histological criteria. The lack of clinical information when identifying cases from laboratory records was judged not to be a
significant factor as the diagnoses recorded in the veterinary records also relied primarily on histologic information to identify cases of marine anemia. To be included in this study, the laboratory reports had to specify marine anemia as the final diagnosis. Cases including only a histological description consistent with marine anemia, but failing to specify the disease in the report, were not included. As the clinicians involved in this study did not record a diagnosis of marine anemia unless the diagnosis had been specified by a laboratory, the exclusion of such cases increased the comparability of the clinical and laboratory derived data. Over 745 veterinary records and 492 laboratory reports were available for review.

The final sources of retrospective data were five independent disease surveys that had been conducted under the sponsorship of federal and provincial government agencies between 1988 and 1992 (Brackett et al, 1991; Brackett et al, 1990a; Brackett et al, 1990b; Newbound and Kent, 1991; Kent, 1993) and a 1991-92 survey conducted as part of this study. All of these surveys relied on convenience sampling methods that were based upon accessibility to farms and the cooperation of salmon farmers. As for the previous sources of information, the location, species involved, and date of diagnosis were recorded for all clinically and pathologically supported
diagnoses of marine anemia that were identified during a review of these surveys.

Demographic data

The changing geographic distribution of the chinook salmon industry was documented by examining the operating licences granted to seacage salmon rearing sites from 1987 to 1992. From 1987-90, issuing such licences was the responsibility of the Canadian Department of Fisheries and Oceans. In 1991-92, the B.C. Ministry of Agriculture, Fisheries and Food became the licensing agency. Therefore, both federal and provincial government licences were collected. The licence data were supplemented by referring to a 1992 report on B.C. salmon farming prepared by industry sources (Anon, 1992).

Case finding

A prospective case finding exercise was conducted between April 1991 and December 1992. The purpose of this exercise was to intensively survey regions and individual farms that had not previously been diagnosed positive for marine anemia, and to determine the geographic origin of affected stock and their parents. Clinical cases of the disease were sought on licensed chinook farms in a wide variety of locations along the B.C. coast by convenience sampling methods. Generally, farms were visited at 2-4 week
intervals until the disease was diagnosed at the site. Over 30% (n=20) of all active chinook sites, representing approximately 25% of all operating companies, were visited. Because of inaccessibility, the northern and outer coasts were not included in this portion of the study. As in the retrospective survey, a region or farm was considered positive if one affected fish was found. Despite the potential for false positive diagnoses that resulted from relying on the identification of a single fish to declare a region as positive, this low diagnostic threshold was typical of the criteria used by many British Columbia fish farmers to determine the marine anemia status of their operations.

To be considered a positive case, affected fish were required to display gross pathological features consistent with those reported by Kent et al (1990). Cases were required to demonstrate a proliferation of immature lymphocytes in the caudal kidney and at least one organ other than the spleen, when routinely prepared, hematoxylin and eosin stained tissue sections were examined (Humanson, 1979). Because virtually all of the diagnoses of marine anemia identified in the retrospective portion of the study had been made in moribund, surface catchable salmon, the source of samples in the prospective survey also focused on this sub-population of the seacages. Also recorded for each farm was the hatchery from which the stock originated, the
strain of fish used, and the source of broodstock.

Analysis

The data were subjected to two principal methods of statistical analysis. The Pearson's correlation coefficient \( r \) was used to explore the relationships between the regional proportion of licences, the proportion of diagnostic submissions and the proportion of marine anemia diagnoses per geographic area (Colton, 1974, pp. 207-214). Inferences regarding the statistical significance of calculated \( r \) values were based on table values published in Colton (1974, p. 349). Differences in the total five year regional proportions of marine anemia were compared by conducting a chi-squared test for homogeneity (Snedecor and Cochran, 1980). All statistical procedures were conducted on Statistix 4.0 software (Analytical Software, 1992. 1958 Eldridge Ave. P.O. Box 130204, St.Paul, Minnesota. 55113).

None of the sources of information in this study represented exhaustive surveys of the total susceptible population, and thus estimates of disease prevalence were subject to sampling error. Confidence intervals can be used to describe a range of possible values for the true population prevalence that is consistent with the observed data, given the variability within the sample (Rothman, 1986). The upper 95% confidence limit for an estimated prevalence of 0% marine anemia in a region, was derived
from values provided in Sackett et al (1991). These values were used to describe the highest prevalence of marine anemia possible, based on the number of submissions examined, for regions declared negative for the disease.

Results

Marine anemia was first described on the southern coast of British Columbia in the fall of 1988. Table 2.1 demonstrates the evolving geographic pattern of diagnoses subsequent to these original reports. Initially restricted to isolated farms in the Sechelt Inlet (Figure 2.1), by 1989, marine anemia had been diagnosed at over 20 sites in the southern region of the province. Over the course of the next three years, four of the five major salmon farming regions in B.C. had been classified as positive. The apparent spread of the disease outside of the southern region was first detected during government sponsored disease surveys in 1990. This apparent spread was later seen, as well, in veterinary and laboratory diagnostic submissions. A review of the chinook farm licences revealed that the geographic distribution of the industry was changing at the same time that marine anemia appeared to be spreading. The southern region of the coast held the majority of licensed chinook sites during each of the six years of this study. Generally, the southern region was followed, in order, by the central region, the west coast,
Table 2.1. The changing geographic distribution of reported cases of marine anemia in British Columbia from 1987 to 1992.

<table>
<thead>
<tr>
<th>YEAR</th>
<th>SOUTH COAST</th>
<th>CENTRAL COAST</th>
<th>WEST COAST</th>
<th>NORWEST COAST</th>
<th>NORTH COAST</th>
<th>OUTER COAST</th>
</tr>
</thead>
<tbody>
<tr>
<td>1987</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1988</td>
<td>V L S</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1989</td>
<td>V L S</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1990</td>
<td>V L S</td>
<td>S</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1991</td>
<td>V S</td>
<td>V L S</td>
<td>V L S</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1992</td>
<td>V L S</td>
<td>V S</td>
<td>V S</td>
<td>V S</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

"-" = region in which marine anemia was not detected

"V" = region declared positive based upon veterinary records

"L" = region declared positive based upon diagnostic laboratory records

"S" = region declared positive in disease surveys
the northwest coast and the north coast in terms of
abundance of licensed chinook seasites (Figure 2.2).
Despite this consistent pattern in licence numbers per
region, there was a progressive shift in farm locations
away from the southern region. This shift was evident in
1990 when, for the first time, the central and southern
coast held the same number of licensed chinook production
sites. As the number of chinook licences decreased in the
southern region, the number of licensed farms grew on the
north, west and northwest coasts. Despite the changing
regional pattern of chinook licences, the total proportion
of chinook licences per year for the entire industry
remained relatively stable: from 1987 to 1992, 46.3 +/-
2.1% of all seacage licences issued per year were to
chinook sites.

The changing geographic distribution of the chinook
industry was mirrored by changing patterns of regional
marine anemia surveillance. During 1988-89, disease surveys
concentrated exclusively on the southern region of the
British Columbia coast when looking for marine anemia. The
first surveys that collected samples outside of this region
did not occur until 1990, coincident with the first report
of the disease on the central region. From 1990 to 1992,
surveyors expanded their investigations to include all
regions except the outer coast. By 1992, only the north and
outer coasts failed to be classified as positive regions.
Figure 2.2. Annual number of British Columbia chinook salmon (*Oncorhynchus tshawytscha*) farm licences issued per region.
The pattern of diagnostic submissions reflected the evolving geographic distribution of the chinook industry (Table 2.2). There was a high positive correlation between the proportion of chinook farm licences per region and the proportion of chinook submissions per region in the veterinary records ($r=0.89$, $p<0.01$). A similar relationship was seen for laboratory submissions ($r=0.82$, $p<0.01$). There was also a high positive correlation between the proportion of chinook samples submitted and the proportion of submissions classified as marine anemia in a region in both veterinary and laboratory diagnostic records ($r=0.71$ and $0.69$ respectively, $p<0.01$ in both cases). Although the number of marine anemia cases seen in veterinary and laboratory records increased in later years, there was no significant difference in the proportion of submissions diagnosed as marine anemia from 1988 to 1992 (chi-squared $= 6.88$, $p = 0.14$) (Figure 2.3). The higher number of positive submissions seen in 1991 coincided with intense public and industry interest in the disease that occurred in the winter of that year.

Upon collapsing the yearly distributions of marine anemia diagnoses between 1987 and 1992 into a five year regional summary (Table 2.3), no statistically significant differences were found among the proportions of marine anemia diagnosed per region ($p=0.94$). Between 1987 and 1992, the disease was diagnosed in, on average, 2.5 +/-
Table 2.2. Proportion of total chinook submissions by region and by year according to veterinary clinical records from 1987-1992.

<table>
<thead>
<tr>
<th>YEAR</th>
<th>N</th>
<th>SOUTH COAST</th>
<th>CENTRAL COAST</th>
<th>WEST COAST</th>
<th>NORWEST COAST</th>
<th>NORTH COAST</th>
<th>OUTER COAST</th>
</tr>
</thead>
<tbody>
<tr>
<td>1987</td>
<td>32</td>
<td>0.53</td>
<td>0.38</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
<td>0.0</td>
</tr>
<tr>
<td>1988</td>
<td>201</td>
<td>0.55</td>
<td>0.30</td>
<td>0.10</td>
<td>0.02</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>1989</td>
<td>196</td>
<td>0.42</td>
<td>0.37</td>
<td>0.11</td>
<td>0.04</td>
<td>0.04</td>
<td>0.02</td>
</tr>
<tr>
<td>1990</td>
<td>77</td>
<td>0.31</td>
<td>0.29</td>
<td>0.27</td>
<td>0.07</td>
<td>0.07</td>
<td>0.0</td>
</tr>
<tr>
<td>1991</td>
<td>133</td>
<td>0.24</td>
<td>0.41</td>
<td>0.27</td>
<td>0.08</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>1992</td>
<td>137</td>
<td>0.28</td>
<td>0.23</td>
<td>0.40</td>
<td>0.12</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>
Figure 2.3. Proportion of laboratory and clinical submissions classified as positive for marine anemia.
Table 2.3. Marine anemia status of all diagnostic submissions according to veterinary records for 1987-1992.

<table>
<thead>
<tr>
<th></th>
<th>SOUTH COAST</th>
<th>CENTRAL COAST</th>
<th>WEST COAST</th>
<th>NORWEST COAST</th>
<th>NORTH COAST</th>
<th>OUTER COAST</th>
</tr>
</thead>
<tbody>
<tr>
<td>MARINE ANEMIA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>POSITIVE</td>
<td>9</td>
<td>6</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MARINE ANEMIA</td>
<td>278</td>
<td>234</td>
<td>149</td>
<td>43</td>
<td>17</td>
<td>6</td>
</tr>
<tr>
<td>NEGATIVE</td>
<td>287</td>
<td>240</td>
<td>152</td>
<td>44</td>
<td>17</td>
<td>6</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OVERALL PREVALENCE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

|                  | 3.1%        | 2.5%          | 2.0%       | 2.3%          | 0           | 0           |
0.4% of the veterinary submissions from each of the regions surveyed.

From 1987 to 1989, farms on the central coast contributed a relatively large proportion of submissions but failed to reveal any cases of marine anemia (Tables 2.2 and 2.3). Table 2.4 provides the highest possible value for the prevalence of marine anemia in regions where no disease was found, given the number of samples collected from each region. Because of the number of submissions received, none of the regions classified as negative were subjected to intense enough sampling to be 95% confident that the disease was not present at or below the 4% level. To be confident, with 95% certainty, that the prevalence of marine anemia in a region was less than 2%, one would need to find no positive cases in no less than 150 submissions (Sackett et al., 1991). None of the regions classified as negative exceeded 73 submissions per year.

As in the retrospective portion of this project, the prospective case finding study revealed cases of marine anemia in all regions sampled. In addition, all of the twenty farms that were visited were declared positive for the disease. In some cases, the disease was diagnosed on the first visit to the farm, whereas others required biweekly visits for three months before a case of marine anemia could be diagnosed.

The results of the prospective portion of this study
Table 2.4. Highest possible true prevalence of marine anemia for regional samples detecting 0% marine anemia (based on 95% confidence levels for estimates of prevalence as in Sackett et al, 1991, p. 176)

<table>
<thead>
<tr>
<th>YEAR</th>
<th>SOUTH COAST</th>
<th>CENTRAL COAST</th>
<th>WEST COAST</th>
<th>NORWEST COAST</th>
<th>NORTH COAST</th>
<th>OUTER COAST</th>
</tr>
</thead>
<tbody>
<tr>
<td>1987</td>
<td>18%</td>
<td>26%</td>
<td>95%</td>
<td>95%</td>
<td>95%</td>
<td>100%</td>
</tr>
<tr>
<td>1988</td>
<td>+</td>
<td>5%</td>
<td>14%</td>
<td>53%</td>
<td>53%</td>
<td>78%</td>
</tr>
<tr>
<td>1989</td>
<td>+</td>
<td>4%</td>
<td>14%</td>
<td>31%</td>
<td>31%</td>
<td>53%</td>
</tr>
<tr>
<td>1990</td>
<td>+</td>
<td>14%</td>
<td>14%</td>
<td>45%</td>
<td>45%</td>
<td>100%</td>
</tr>
<tr>
<td>1991</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>26%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>1992</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

"+" = samples demonstrating > 0% marine anemia.
failed to demonstrate a consistent pattern for the origins of salmon affected with marine anemia. No single hatchery or strain of chinook could be identified as the exclusive source of affected fish. The broodstock of positive progeny identified in this study came from commercial and federal government hatcheries throughout Vancouver Island and the southern coast of B.C. Wild stocks from northern Vancouver Island and the Yukon also provided progeny that were later diagnosed with marine anemia. However, as broodstock were not examined during this study, the distribution of marine anemia in this sub-population cannot be described.

Discussion

A study of the evolving geographic distribution of a disease can provide important clues as to the epidemiology and etiology of the disease involved (Schwabe et al., 1977). For this reason, descriptive epidemiological studies are often used to attempt to define the changing geographic distribution of a disease (Schwabe et al., 1977). However, descriptive data can sometimes lead to biased impressions of the nature of the disorder studied (Kelsey et al., 1986). Without paying due attention to the potential biases affecting studies of the distribution of marine anemia, investigators could easily be misled.

The review of diagnostic submissions appeared, at first, to support the hypothesis that marine anemia was
originally limited geographically to the south coast, and then spread to other fish farming regions. However, this interpretation failed to consider the underlying changes in the demographics of the susceptible population. Once the spatial pattern of a disease has been established, it is generally recommended that the geographic characteristics of the susceptible population be studied and considered before epidemiologic hypotheses are generated (Martin et al., 1987, pp. 97-98; Schwabe et al., 1977). Failure to do this generates the risk of encountering a "demographic bias" which can occur when the specification and selection of a study sample fails to take into account the demographic characteristics of the general population at risk. This failure was the primary source of bias in the biogeographic study of marine anemia.

The principal question of concern in this investigation was, "Can it be confidently stated that marine anemia spread throughout the major salmon farming regions of B.C.?" To answer this question one must correctly classify a region, on a yearly basis, as positive or negative with respect to the disease. The potential misclassification of the marine anemia status of a group limited our ability to answer this question. The requirement of identifying only a single positive fish to declared a farm or region as positive for marine anemia created a risk of false positive diagnosis. As the validity
and reliability of the criteria used to diagnose marine anemia are unknown, the repeated testing of the same farms and regions increased the probability of achieving a false diagnosis. Alternatively, the small number of submissions received from some regions created a significant potential for missing cases of the disease. Based on published numbers of chinook salmon farmed per year (Anon, 1992), and considering an average of five salmon subjected to histologic examination per diagnostic submission, approximately 0.006% of the susceptible salmon population was examined for marine anemia. In general, too few diagnostic submissions were received from most of the regions to confidently rule-out the presence of marine anemia.

There were substantial changes in the regional distribution of chinook farms on the B.C. coast from 1987 to 1992. These changes had a significant influence on the perceived pattern of spread of marine anemia. As the proportion of licensed farms in an area increased, so did the number and proportion of diagnostic samples taken from the region. With this increased intensity of sampling, on a regional basis, came increased numbers of positive diagnoses. This relationship is consistent with the theories of disease sampling (Richards, 1982). Because of a concern for confidentiality, most laboratory submissions did not provide sufficient information with which to
exclude the possibility that the increase in the number of submissions seen in a region was not due to an increased rate of submission from only one or two farms. However, the clinical records suggested that this was not the case.

Because prevalence is a fraction, as more fish are examined, a larger number of cases will tend to be identified. For a sample to identify a specified number of cases, a certain proportion of the population must be sampled. Rarely was a sufficiently large number of samples submitted from a region to confidently rule out the presence of marine anemia at levels less than 10% of the total submissions. Because the prevalence of the disease in submissions from each region appeared to be low (2.5%), there exists the possibility that all regions classified in previous years as negative, may have been mis-classified.

Others have previously proposed that the detection of marine anemia outside of the Sechelt area may not have been due to the spread of the disease, but rather to a lack of previous diagnosis of an existing condition (Newbound and Kent, 1991). When dealing with a disease of low prevalence, it is invalid to declare a region as unaffected by a disease when no cases are found, especially if unequal sampling protocols have been applied or too small a proportion of the population has been sampled (Richards, 1982). This appears to have been the case for marine anemia. Alternatively, it may be hypothesized that, as new
areas for salmon farming opened up, the disease followed by quickly spreading into newly introduced susceptible populations. However, the isolation of farms and lack of contact between farms reduces the likelihood that a newly introduced disease could spread as rapidly over as large a region as the southern coast of British Columbia.

Unbiased samples, collected by a formal random sampling scheme, are generally recommended for investigations designed to determine the prevalence of a specific disease in a population (Hancock et al, 1988). None of the retrospective data used in this study were derived from any form of random sampling. Indeed, the disease surveys, and the veterinary and laboratory submissions were all biased by the knowledge of the disease status of a region or farm. Areas considered positive for the disease were generally sampled more intensively than areas thought to be negative (Brackett et al, 1990a). This was likely a reflection of regional differences in fish farmer concern, and the emphasis of early researchers to study the disease at the individual fish or pen level and not at the regional level. A sampling scheme that has a random component and samples only a portion of the population at frequent intervals, is a more effective means of monitoring a disease than those that expend all resources on a single large scale census (Farver et al, 1985). The potential regional and farm level mis-
classifications of marine anemia status encountered in this study emphasize the potential danger of relying on single, opportunistic visits to determine the disease status of a larger demographic group.

The results of this study suggest that the apparent spread of marine anemia was due to changing regional disease surveillance efforts that arose from the shifting distribution of the susceptible population. The ubiquitous nature of marine anemia, as seen in the prospective portion of the study, and the lack of a documented spread of the disease suggest that marine anemia is an endemic problem in B.C. farmed chinook salmon and not a spreading epidemic. Identifying marine anemia as an endemic instead of an epidemic disease is an important distinction. The three commonly recognized patterns of disease occurrence (sporadic, endemic and epidemic) are the result of different critical ecological and pathological factors. (Martin et al., 1987, p.108; Kelsey et al., 1986). Whereas endemic diseases are usually the result of a predictable, long-term balance between the host, environment, and pathogenic agent, epidemic diseases reflect a major imbalance that favours the agent (Martin et al., 1987, p. 108). A clear understanding of the pattern of occurrence of marine anemia is therefore a critical first step in the development of a rational basis for disease management recommendations.
References


CHAPTER 3

AN EVALUATION OF SURFACE MORIBUND SALMON AS INDICATORS OF SEAPEN DISEASE STATUS

Introduction

Samples of fish are frequently collected from salmon netpens in order to estimate the nature and severity of disease in the general seafarm population. The precision of these estimates can be described and their biases minimized if random sampling methods are employed (Thorburn, 1992). It is, however, difficult to sample a seapen so that all fish have an equal, non-zero probability of capture without creating significant handling stresses for the population. For this reason, fish health investigators often rely on samples obtained from readily accessible segments of the pen population (Brackett et al., 1991). The two most accessible segments of the pen population are moribund fish captured at the surface of the water, and dead fish retrieved from the bottom of the pen by scuba diving. However, reliance on microscopic and microbiologic criteria for the diagnosis of many salmon diseases (Kent, 1992; Pillay, 1990) has led investigators to focus their diagnostic efforts on fish captured alive (Pettijohn, 1983;
McDaniel, 1975). In seapen aquaculture, adherence to this advice has led to a dependence on fish that are "catchable" at the surface of the water as the primary source of diagnostic samples obtained during disease investigations (Brackett et al., 1991).

In fish culture facilities where water current is swift enough to cause segregation, fish will tend to separate according to general health status (Kabata, 1985). However, in seapens, where the effect of water flow on swimming behaviour is not clear (Sutterlin et al., 1979), the distribution of moribund salmon is less obvious. It is important to improve our understanding of how the diseases and distribution of moribund seapen salmon are related before extrapolating observations based on non-probability samples to the general population. The purpose of this study is to describe the moribund sub-population of salmon in commercial seapens in order to determine if the patterns of disease observed in these surface catchable fish (SCF) are representative of the entire pen.

Materials and Methods
Setting and design of mark and recapture trials

This study was conducted on commercial chinook salmon (Oncorhynchus tshawytscha) seafarms in southern British Columbia, Canada between 1991 and 1993. The ages of fish observed ranged from nine-month old smolts to four-year old
broodstock. Fish were housed in 3500m$^3$ or 2500m$^3$ seacages that were roughly cubical in shape. Pen depth ranged from 15-20m. Fish numbers per pen varied from approximately 12,000 smolts to 1500 four-year old fish. Participating farms were selected on the basis of convenient access and owner co-operation.

Modified mark and recapture trials were conducted at six commercial salmon farms. Four adjacent pens of chinook salmon of the same year-class were selected at each participating farm. Trials were four days in length, with follow-up visits conducted by telephone or in person at two and four week intervals.

All dead fish were removed from the seapens at the start of each trial. Dipnets were used to catch moribund salmon from the pens on the first, second and third days. The length of the dipnets restricted capture efforts to a zone 1-2m from the netpen edge and to a depth of 1-1.5 m. This capture area was similar to that used by veterinarians and fish farm personnel when collecting live fish for diagnostic tests. All pens were exposed to capture efforts of equal intensity.

The captured salmon were subjected to a brief external examination prior to being tagged. A uniquely identified Floy anchor ("spaghetti") tag was then inserted behind the dorsal fin, with a Mark II tagging gun (Floy Tag and Manufacturing, Inc. Seattle, Wash.), anchoring the tag in
the epaxial musculature. This location allowed the tag to be visible from the surface and from under water. The date and time of tagging were recorded for each fish. After examination and tagging, fish were released back into their pen. The duration of the examination and marking procedure was always less than one minute. Daily scuba dives were conducted to remove all mortalities from the pens on days two, three and four. Tagged fish were recovered when found dead. Fish farm staff were asked to record the date and identification of any tagged dead fish found for one month after the end of the trial.

Pathological evaluation

Gross post-mortem examinations were conducted on all dead fish. Each fish was assigned a body condition score that ranged from 1 to 4. Based on abdominal fat depots, a score of 1 was given to severely emaciated fish, while a score of 4 was recorded for a fish in excellent body condition. The fish were also categorized according to the predominant pathologic process observed at necropsy. Four diagnostic categories were created. These categories were created as an *a priori* means to facilitate on-farm classification of the disease status of fish by categorizing them on the basis of the predominant gross pathological changes observed at necropsy. The four categories were: (1) *Productive processes*. This category
was reserved for fish exhibiting fibrinous or granulomatous processes at gross necropsy; (2) Non-productive processes. This category was reserved for fish suspected of having infectious diseases but lacking fibrinous or granulomatous reactions. Fish exhibiting typical signs of Gram-negative infections (cutaneous erythema, visceral petechiation and congestion {Kent, 1992}), inflammation of the intestinal tract, non-suppurative meningitis, or rickettsial septicemia were included in this category. Although rickettsial septicemia in salmon is characterized by pyogranulomas, these lesions are restricted to the liver and are not as exhuberent as other granulomatous processes that, for the purposes of this study, were classified as productive infections. Fish exhibiting visceral organ enlargement, without signs of other infectious processes were also placed in this category as these signs have been associated with a number of proven or potentially infectious disorders of seapen salmon such as plasmacytoid leukemia, microsporidial infection and systemic diplomonad infections {Kent, 1992}; (3) Non-infectious processes. This category was reserved for cases of gastric bloat, victims of algae blooms or predators, precocial maturing fish and non-smolts (runts); (4) Open. Dead salmon that could not be placed into one of the three categories described above, were recorded as open diagnoses.

A sub-sample of 75 mortalities selected by convenience
were subjected to histological examination of routinely prepared hematoxylin and eosin stained sections of multiple visceral organs (Humanson, 1979) to help validate the diagnostic categories described above. Table 3.1 illustrates how some of the commonly diagnosed diseases of seapen chinook salmon in B.C. (Kent, 1992; Brackett et al, 1991; Hicks, 1989) would be categorized according to this system of disease classification.

Analysis of the tagging trials was conducted on a statistical software package (Statistix 4.0, Analytical software, St. Paul, Minnesota). A chi-squared test for several proportions (Colton, 1974, pp. 179-181) was applied to the body condition and gross necropsy data to test for significant differences between the tagged and untagged groups of mortalities. Relationships between the rate of SCF capture, the pen mortality rate, and the ratio of tagged to total mortalities were explored by calculating Pearson's correlation coefficients (r) (Colton, 1974, pp. 207-214). Statistical inferences regarding the significance of the r-values were provided by referring to table values published in Colton (1974, p. 349).

In addition to the "mark and recapture" trials, SCFs were collected for clinical and pathological examination during 36 one day-long visits involving twelve production sites. At each of the visits, all pens on the sites containing chinook salmon were searched from the surface
Table 3.1. Examples of diseases commonly diagnosed in chinook seapens in British Columbia\textsuperscript{a} classified according to the diagnostic categories developed for this study

<table>
<thead>
<tr>
<th>NON-PRODUCTIVE PROCESSES</th>
<th>NON-INFECTIONOUS PROCESSES</th>
<th>PRODUCTIVE PROCESSES</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Vibriosis</td>
<td>- Waterbelly (Bloat)</td>
<td>- Bacterial Kidney Disease</td>
</tr>
<tr>
<td>- Plasmacytoid Leukemia</td>
<td>- Non-smolt (Runt)</td>
<td></td>
</tr>
<tr>
<td>- Rickettsial Septicemia</td>
<td>- Precocial Maturation</td>
<td></td>
</tr>
<tr>
<td>- Systemic Diplomonad Infection</td>
<td>- Algal Bloom</td>
<td></td>
</tr>
<tr>
<td>- Myxobacteriosis</td>
<td>- Predation</td>
<td></td>
</tr>
<tr>
<td>- \textit{Loma salmonae} Infection</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a}: (Kent, 1992; Hicks, 1989, Brackett, 1991)
for catchable fish. Fish were again caught by dipnet from walkways surrounding the pens. After capture, fish were euthanized by cervical severance, and visually examined for gross external or internal signs of disease. A sub-sample of SCFs was also subjected to histologic examination as outlined above.

Behavioural observations

Information about the behaviour, location, and movement of SCFs was obtained by surface and underwater observations. These observations were collected during farm visits and tagging trials. Field observations were structured to determine the location of moribund fish in the seapens, to document their movements throughout the pen, and to observe the relationship of SCFs with other moribund fish, as well as with the "healthy" main group of salmon in the seacages. Moribund fish were identified by the presence of external lesions, behavioural changes and/or abnormal swimming patterns. By combining observations of external features and tag status, the activity of individual fish could frequently be traced throughout the pens. Surface observations were made from walkways surrounding the seacages for 30-45 minutes per pen per visit. Underwater observations that lasted approximately 20-30 minutes were made during scuba dives in the pens. During this time, the investigator made a slow
descent to the pen bottom, remained at the bottom for most of the dive, and then slowly ascended to the surface. To encourage consistent descriptions of the dynamics of moribund fish in the netpens, all surface observations plus underwater observations in all pens in the tagging trial and approximately 75% of the day-visits pens, were made by the primary investigator.

Results

Table 3.2 shows the mortality rate of tagged fish. The majority (62%) of the tagged salmon died within the first 24 hours post-tagging. By day three, 72% of tagged fish had died. Follow-up visits revealed that 25% of the fish not accounted for by the fourth day of the trial were later found dead, averaging a 2-week post-tagging survival time. Some marked fish survived for over one month. Salmon that survived for over 2 weeks were often dark and emaciated when recovered. The remaining fish in the trial (21%) were lost to follow-up post-tagging. Tag loss, as a reason for loss to follow-up, could only be documented in one case. In addition, post-trial farm visits suggested that the crew at two of the sites failed to adequately monitor mortalities after the fourth day of the trial, thus allowing some tagged mortalities to go undetected.

The majority (82%) of examined SCFs demonstrated obvious pathological and clinical abnormalities. Of the 187
Table 3.2. Post-tagging survival time of moribund salmon captured at the surface of seapens.

<table>
<thead>
<tr>
<th>SURVIVAL TIME</th>
<th>PERCENT</th>
<th>NUMBER</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 24 hours</td>
<td>23</td>
<td>43</td>
</tr>
<tr>
<td>24 hours</td>
<td>39</td>
<td>72</td>
</tr>
<tr>
<td>48 hours</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>72 hours</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>&gt; 72 hours</td>
<td>28</td>
<td>53</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>100</strong></td>
<td><strong>187</strong></td>
</tr>
</tbody>
</table>
SCFs clinically examined during the tagging trial, only 18% failed to demonstrate external lesions or behavioural abnormalities. External changes were visible in 65% of the SCFs. The general classes of external signs were: ocular lesions (30%), cutaneous lesions (18%), darkened colouration (15%), small, emaciated fish (15%), development of secondary sexual characteristics (10%), abdominal swelling (6%), scoliosis/kyphosis (4%) and miscellaneous lesions (2%). Behavioural abnormalities such as bumping into the nets, circling or spiralling, swimming apart from the main group, prolonged stays at the surface of the water, and mouth-breathing were observed in fish with and without external lesions. The 18% of SCFs failing to demonstrate clinical signs could only be distinguished from apparently healthy fish by their tendency to swim alone at the surface and by their catchability. Not all fish observed at the water surface, however, could be caught. Fish with obvious clinical abnormalities were frequently able to evade capture, as were clinically normal fish seen at the surface. Pathological evidence, in the form of gross and histologic lesions, revealed that 95% of the 366 SCFs examined during regular farm visits and tagging trials exhibited pathological abnormalities.

Significant differences (p=0.002) were seen in the diagnostic categorization of tagged and untagged mortalities using the classification system created for
this study (Table 3.3). Non-productive and non-infectious processes were more often diagnosed in untagged mortalities, while productive processes and open diagnoses were more frequent in tagged mortalities. In some of the trials, estimates of the proportional mortality attributable to specific disease categories, such as gastric bloat and non-smolts, differed by over 30% in the tagged and untagged groups.

Although there was no significant difference in the mean body scores of tagged and untagged dead fish in any of the trials, there was a trend for the tagged mortalities to be in worse body condition than the untagged group. This trend proved to be significantly different in three of the six trials (Table 3.4). However, when the body score data were pooled for all six trials, there was no significant difference in the body condition scores for the tagged versus untagged mortalities (p=0.24). When body condition data were reviewed for specific disease categories, 81% of the untagged mortalities classified as non-infectious deaths were found to be in good body condition compared to only 32% in tagged mortalities (p<0.0001).

The rate of SCF capture was generally low. On average, less than one fish could be caught by dipnet from a pen for every hour of searching (range = 0.7-8.0 SCFs caught/pen/hour). The daily rate of SCF capture was highly correlated with the daily mortality rate (r = 0.91, p =
Table 3.3. Proportional mortality of marked and unmarked moribund seapen reared salmon.

<table>
<thead>
<tr>
<th>DIAGNOSTIC CATEGORY</th>
<th>UNTAGGED MORTALITIES</th>
<th>TAGGED MORTALITIES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>N (%)</td>
</tr>
<tr>
<td>Non-productive</td>
<td>303 (52%)</td>
<td>51 (35%)</td>
</tr>
<tr>
<td>infections</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-infectious</td>
<td>140 (24%)</td>
<td>22 (15%)</td>
</tr>
<tr>
<td>disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Productive</td>
<td>82 (14%)</td>
<td>42 (29%)</td>
</tr>
<tr>
<td>infections</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Open</td>
<td>58 (10%)</td>
<td>30 (21%)</td>
</tr>
<tr>
<td>Total</td>
<td>583 (100%)</td>
<td>145 (100%)</td>
</tr>
</tbody>
</table>
Table 3.4. Distribution of body condition scores of moribund fish recovered in tagging trials.

<table>
<thead>
<tr>
<th>FARM</th>
<th>BODY SCORE</th>
<th>UNTAGGED FISH</th>
<th>TAGGED FISH</th>
<th>P-VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>16</td>
<td>21</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>27</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>29</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>34</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>13</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>44</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>40</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>1</td>
<td>16</td>
<td>10</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>33</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>32</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>19</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>1</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>1</td>
<td>12</td>
<td>0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>47</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>29</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>12</td>
<td>19</td>
<td></td>
</tr>
</tbody>
</table>
<0.05). However, the proportion of SCFs captured relative to the total number of salmon in the pen was always low. On average only 0.16 fish were caught for every 1000 salmon in a pen (range = 0.06-1.25). The mean number of SCFs captured relative to the number of mortalities recovered daily was 3.6 SCFs per 10 mortalities (range = 0.7-8.8). With increasing mortality rate, a declining number of SCFs was caught relative to the number of mortalities recovered. The majority of mortalities died without being tagged. On average, only 17% of the mortalities recovered each day were tagged fish (range = 5%-38%). As mortality rates increased, there was a tendency for the proportion of mortalities that were tagged to decrease.

Surface and underwater observations revealed a consistent pattern of distribution of salmon within the seapens. Isolated, slow swimming fish were visible in the first 1-2 m depth, in the centre and at the edges of the pens. This was the group of fish from which SCFs were obtained. Next, a band of salmon could be found at a depth of approximately 2-5m. This group could frequently be visualized from the surface. When viewed from above, the salmon in this top band swam as a group in a circular pattern. Isolated fish were seen to be swimming slowly at the edges of this group. These slow swimmers often exhibited external lesions or behavioural abnormalities. However fish with obvious external lesions could also be
seen swimming within the top band of salmon. The majority of the fish in the pen were usually found at depths greater than 7m and therefore could rarely be seen from the surface. The specific shape of this aggregate of salmon varied with the shape of the pen, but in general, the fish in this layer tended to swim in a single large group or in a number of smaller groups. All fish within a group, however, shared the same swimming direction and speed. Fish could be seen to move between the top band and the deeper main group. Salmon with obvious clinical signs were observed within this lower main group, while slow swimming isolated fish, many with obvious clinical abnormalities, swam at its periphery. Small or clinically abnormal fish could also be found at the bottom of the lower main group. The final group of salmon encountered during every dive was the pile of dead and dying fish located at the bottom of the pen.

After being released post-tagging, SCFs were observed to move throughout the pen. At times, tagged fish joined the top band or the lower group of salmon. However, the majority of tagged SCFs were seen either at the periphery of the groups, at the surface of the water, or at the edges and corners of the pen. As a general rule, tagged fish tended to be swimming in isolation, at slower speeds, and often in a different direction, than their apparently healthy cohorts.
Species other than the farmed salmon, such as shiner perch (*Cymatogaster aggregata*), lingcod (*Ophiodon elongatus*), Pacific herring (*Clupea harengus pallasi*), and Pacific dogfish (*Squalus acanthias*) were often found within the netpens. None of these species were ever seen within the top band or lower group of salmon. Like many of the moribund salmon, these species tended to frequent the corners of the pen or swam at the periphery of the healthy groups of salmon.

**Discussion**

Surface catchable fish (SCFs) are an unhealthy sub-population of chinook salmon in commercial seapens. The high mortality rate documented in these fish confirmed that salmon captured from the surface of a seapen are indeed moribund. Although the presence of human observers may have affected the distribution of the salmon within the pen, the results of this study could not substantiate the hypothesis that seapen salmon, once becoming ill, will leave the main group of salmon, move to the surface, and stay there a few days before dying. Instead, it appears that moribund fish can be found throughout the netpen and that many of these fish die without becoming accessible for surface sampling. If significant differences between the disease pattern of SCFs and moribund salmon found elsewhere in a seapen exist, generalizations of disease information from SCFs to the
entire pen population would be invalidated (Rothman, 1986). The results of this study suggest that such differences can exist.

Few, if any, living organisms or diseases are randomly dispersed in nature (Taylor et al, 1978; Anderson and May, 1991). Because social behaviour can be affected by disease status (Safriel, 1982; Schmid-Hempel and Muller, 1991) and disease prevalence can be remarkably different within specific sub-groups in animal populations (Tessaro et al, 1993), diagnostic samples that are based on specific sub-groups are unlikely to present a true picture of the nature of disease for the entire reference population. Based on our findings, there are two important ways by which data derived from SCFs can generate biased estimates of the disease status of a chinook seapen: (1) by providing an inadequate sample size, and (2) by providing an unrepresentative spectrum of disease.

The small number of moribund fish that can be caught at the surface with a reasonable amount of effort limits the reliability of investigations based solely on the examination of SCFs. Regardless of the pen mortality rate, the proportion of SCFs caught relative to the entire pen population was always small. The probability of detecting disease in a sample is affected by the size of the source population and the size of the sample (Cannon and Roe, 1982). Sampling theory would therefore dictate that, on
average, only diseases of high prevalence in netpens can be readily detected by sampling SCFs. A similar problem exists when data collected from SCFs is extrapolated to the overall mortality pattern in a pen. The low mean ratio of tagged to total mortalities indicates that SCFs generally represent a small proportion of the total number of fatally ill fish in a pen. Diseases responsible for the majority of mortality in a seapen may therefore be seriously underrepresented in samples of SCFs.

Estimates of disease prevalence in seapens based on SCF samples may be further biased if certain disorders have a higher probability of being detected in surface samples than do other diseases. Aspects of the natural history of chinook (Healy, 1991) and previous studies of swimming behaviour in pen-reared salmon (Sutterlin et al, 1979) suggest that the distribution of salmon in seapens represents neither true schooling behaviour nor true random dispersal. Instead, it has been suggested that the swimming patterns and distribution of salmon in a pen is the result of physiological demands, and the desire to optimize distance from both nearest neighbours and the pen edge (Sutterlin et al, 1979). As the severity and clinical course of a disease can affect an animal's ability to meet physiological and production requirements (Blood et al, 1983), differential effects of different diseases could conceivably influence the location of sick salmon within a
pen. As the probability of surface capture is affected by the amount of time a fish spends at the surface, differential effects of disease on the position of fish in a pen would influence the pattern of disease seen in surveys of SCFs. Fish affected by chronic disease processes would be less able to meet the demands of staying within the "school" before death and would, therefore, move to the periphery of the main groups, thereby increasing the probability of capture. Alternatively, fish dying acutely or peracutely would spend little time at the margins of the population before death and would therefore be less accessible for surface capture. This hypothesized relationship of disease status and fish distribution is supported by the trends in body condition scores and the differences in proportional mortality patterns seen in the tagged and untagged groups of fish. Chronic conditions such as bacterial kidney disease and non-smolts (Ferguson, 1989) predominated in the tagged mortalities, while diseases resulting in more acute deaths, such as vibriosis (Roberts, 1978), were more commonly diagnosed in untagged mortalities. Studies demonstrating that competitive ability and anatomical characteristics affect the aggregative behaviour of various fish species (Ranta et al, 1993; Ranta et al, 1992) are further evidence in support of the hypothesis that the clinical course and severity of a disease can affect the location of a sick fish relative to
its healthy cohort, and therefore its accessibility for surface sampling.

Moribund fish captured at the surface of a pen are frequently used as the primary source of information from which the clinical features of "new" seapen diseases are described (for example see, Kent et al, 1990; Speare et al, 1989; Hogans, 1989). As tagging apparently does not significantly alter the final clinical course of disease in marked fish (Eames and Hino, 1983), the vast majority of SCFs were in the terminal stages of their disease. Therefore, a case series collected from the surface may not represent the entire spectrum of clinical effects of a disease. An important limitation to SCF samples, therefore, is the potential failure of the sample to include less severely affected, or recovering fish, thus leading the investigator to conclude that the effects of the disease in question are manifested primarily through mortality. Such conclusions would be in contrast to many other farm animal diseases where important population effects are more often the result of the large number of sub-clinically or less severely affected animals than the smaller number that die (Martin et al, 1987).

SCFs will likely remain the standard source of live, sick salmon from seapens because practical limitations prevent the selective capture of non-surface moribund fish for routine disease monitoring programs. Random samples are
an alternative to the non-probability sampling of SCFs. However, random sampling requires the manipulation of the entire population as well as the sacrifice of healthy fish, neither of which is agreeable to most fish farm owners on a routine basis. The results of this study indicate that future research efforts should be directed towards generating useful disease information about seapens from sources other than the evaluation of surface moribund fish alone. Other researchers have suggested that behavioural observations are potentially valuable tools for monitoring the changing disease pattern in salmon seapens (Sutterlin et al., 1979). Because mortality, as opposed to sub-clinical effects of disease, is a primary concern of B.C. salmon farmers (Anon, 1992), special efforts should be taken to determine the value of performing routine necropsies on the dead fish found at the pen bottom as a means of monitoring the mortality pattern of seapen salmon.

Routine necropsies are seldomly exploited as a tool for disease investigation or surveillance on fish farms. Experience in other animal production industries has demonstrated that valuable information can be gained by conducting regular post-mortem examinations (Kelly, 1984; Ribble, 1992). The rapid autolysis and post-mortem contamination of dead fish have been considered major factors limiting the use of routine necropsies on salmon farms (Brackett et al., 1990; Kent, 1992). Strategies that
include the frequent recovery of dead fish and the use of disease categories based on gross pathologic changes would help to overcome these perceived limitations of necropsy series, and thus allow for the direct assessment of changing mortality patterns on sea farms.
References


Appendix 3.1. Morbidity and mortality characteristics of farms participating in the tagging trials.

<table>
<thead>
<tr>
<th></th>
<th>FARM A</th>
<th>FARM B</th>
<th>FARM C</th>
<th>FARM D</th>
<th>FARM E</th>
<th>FARM F</th>
</tr>
</thead>
<tbody>
<tr>
<td>% SCF dead in 24 hours</td>
<td>43</td>
<td>54</td>
<td>76</td>
<td>NA</td>
<td>62</td>
<td>60</td>
</tr>
<tr>
<td>% dead fish with tags</td>
<td>38</td>
<td>34</td>
<td>12</td>
<td>6</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>Productive infection</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>33</td>
<td>31</td>
<td>3</td>
</tr>
<tr>
<td>(% tagged)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Productive infection</td>
<td>0</td>
<td>4</td>
<td>16</td>
<td>39</td>
<td>51</td>
<td>4</td>
</tr>
<tr>
<td>(% untagged)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-productive infection</td>
<td>0</td>
<td>68</td>
<td>83</td>
<td>11</td>
<td>51</td>
<td>61</td>
</tr>
<tr>
<td>(% tagged)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-productive infection</td>
<td>0</td>
<td>46</td>
<td>48</td>
<td>42</td>
<td>31</td>
<td>50</td>
</tr>
<tr>
<td>(% untagged)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-infectious (% tagged)</td>
<td>71</td>
<td>13</td>
<td>6</td>
<td>44</td>
<td>6</td>
<td>28</td>
</tr>
<tr>
<td>Non-infectious (% untagged)</td>
<td>50</td>
<td>46</td>
<td>21</td>
<td>14</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>Open (% tagged)</td>
<td>29</td>
<td>11</td>
<td>11</td>
<td>11</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>% Open (% untagged)</td>
<td>50</td>
<td>4</td>
<td>14</td>
<td>28</td>
<td>13</td>
<td>35</td>
</tr>
</tbody>
</table>
CHAPTER 4

OBSERVER VARIATION IN THE HISTOLOGIC DIAGNOSIS OF PLASMACYTOID LEUKEMIA (MARINE ANEMIA)

Introduction

Plasmacytoid leukemia is an apparently infectious neoplastic disease that has recently been identified in farmed salmon in British Columbia, Canada. The disease is characterized by a massive infiltration of visceral organs, retrobulbar tissues, muscle, and other structures by immature lymphoblasts resembling plasma cells. The disease, also known as marine anemia, has been associated with large scale epidemic mortality on salmon farms (Kent et al., 1990).

The diagnosis of plasmacytoid leukemia is currently confirmed by histology (Kent, 1992; Brackett et al., 1991a). Several disease surveys of Canadian salmon farms have relied on histologic means to identify the disease (Brackett et al., 1991a; Brackett et al., 1991b; Newbound et al., 1991a). Histology has also been used to define plasmacytoid leukemia in laboratory based research (Eaton et al., 1991; Kent et al., 1990; Newbound et al., 1991b), as
well as in the assessment of new diagnostic tests (Newbound et al., 1993). Histology therefore, can be considered the current "gold standard" for the diagnosis of plasmacytoid leukemia. However, the reliance on routine histologic methods as the definitive test for neoplasms has been questioned (Moulton et al., 1990; Thomson, 1978). Neoplasia of the hematopoietic system in particular can be difficult to diagnose using histopathology alone (Moulton et al., 1990).

The clinical performance of a diagnostic test can be defined as its ability to detect the presence or absence of a disease. It is usually determined by calculating the sensitivity, specificity and predictive values of the test (Ransohoff and Feinstein, 1978). However, to determine these parameters, the true disease status of the patients examined must be known. In the absence of alternative means to determine the true disease status of subjects, measures of diagnostic agreement can be used to assess the precision and validity of a diagnostic method (Martin and Bonnett, 1987; Thompson et al., 1988). By calculating inter- and intra-observer agreement, one can estimate how well a method is measuring what it purports to measure (Martin et al., 1987). Uncovering reasons for disagreement may help to refine the discriminatory ability of the diagnostic procedure and thus improve its diagnostic value (Martin and Bonnett, 1987).
The reliability of epidemiologic studies can be limited by their ability to accurately classify the disease status of individuals or groups (Rothman, 1986). The objective of this study was to assess the level of agreement for the histopathological diagnosis of plasmacytoid leukemia. The study was designed to allow for the exploration of potential reasons for diagnostic disagreement. The results were used to estimate the reliability and comparability of diagnostic information obtained from fish pathology laboratories for epidemiologic studies of plasmacytoid leukemia.

**Materials and Methods**

Selection of samples and participants

A panel of four pathologists was asked to determine the plasmacytoid leukemia status of tissues taken from 50 salmon. Diagnostic material was collected from the fish histopathology files of a single laboratory. Samples were considered for inclusion in the trial if: (1) they had originally been submitted for histological confirmation of plasmacytoid leukemia, (2) the disease status had been previously determined and recorded by the reference pathologist used in this trial, (3) routinely prepared, paraffin processed, hematoxylin and eosin stained tissue sections were available for examination (Humanson, 1979), and (4) at least six of the following tissue sections were
included in the samples: caudal kidney, liver, spleen, pancreas, pyloric ceceae, terminal colon or retrobulbar tissue. Fifty cases were randomly selected from a complete list of over 200 eligible samples by using a random number table. Histologic slides were relabeled to insure blind evaluation.

Participating pathologists had extensive experience diagnosing diseases of farmed Pacific salmon (Oncorhynchus spp.) using histologic methods but varied in their experience with this disease. Experience in diagnosing plasmacytoid leukemia decreased from the reference pathologist (A), who had extensive clinical and experimental experience with the disease, to pathologist D, who had not diagnosed plasmacytoid leukemia prior to the trial. Pathologist B's experience was similar to, yet not as extensive as A, whereas C was intermediate between B and D. When pathologist A originally evaluated the cases, he had access to the clinical history and any pertinent gross pathologic findings for each submission. No other pathologist was given these details. Instead, all participants were informed that the samples had been submitted for confirmation of plasmacytoid leukemia, thus implying a pre-existing degree of clinical suspicion. Because of the different levels of experience in diagnosing the disease, a previously published description of the histologic features of plasmacytoid leukemia (Kent et al,
was provided to all participants.

Diagnostic categories

The pathologists were asked to categorize individual samples as plasmacytoid leukemia-positive (PL+), negative (PL-) or questionable (PL?). A positive diagnosis required the features of the case to be consistent with the description provided, a negative diagnosis required the case to be inconsistent with the description. A questionable diagnosis was defined as a case that had features suggestive of the disease, but not adequately fulfilling the criteria provided. Pathologist A was required to blindly re-evaluate the case series according to these same guidelines. The original diagnoses made by the reference pathologist were the basis for the calculation of intra- and inter-pathologist agreement.

Analysis

Observed agreement was assessed by summing the concordant cells of summary k X k tables (Fleiss, 1981). Overall agreement was determined for 3 x 3 tables which included all diagnostic categories (PL+, PL- and PL?). Agreement for individual categories (PL+, PL- or PL?) was assessed by collapsing the 3 x 3 tables into 2 x 2 tables in which categories other than the one of interest were combined into a single comparison group. Agreement was also
assessed by the kappa statistic. Kappa is a measure of agreement beyond that expected by chance alone and can be interpreted as a measure of the clinical value of an observation (Fleiss, 1981; Kraemer, 1979). Values of kappa > 0.75 were taken to represent excellent agreement, between 0.40 and 0.75 represented fair to good agreement, and values < 0.40 indicated poor agreement beyond chance (Fleiss, 1981). Because the kappa coefficient has been shown to be sensitive to changes in disease prevalence (Thompson et al., 1988), the z-statistic was calculated (Colton, 1974) to determine if significant differences existed between the estimates of disease prevalence made for the test series by each of the pathologists.

Results

Table 4.1 illustrates the inter- and intra-pathologist reproducibility of diagnosis for the three diagnostic categories described. Although there was no statistically significant difference in the mean estimated prevalence of leukemia in the samples (p>0.20), agreement on the classification of individual cases was generally poor. All three pathologists agreed with only 14% of the reference pathologist’s original diagnoses and 19% of his blind evaluations. Overall inter-pathologist kappa values ranged from -0.03 (A versus D) to 0.36 (A versus B). The inter-pathologist agreement with the reference pathologist’s
Intra = intra-pathologist agreement
Inter = mean inter-pathologist agreement with reference pathologist's original diagnoses

<table>
<thead>
<tr>
<th>Kappa</th>
<th>0.15</th>
<th>0.07</th>
<th>0.05</th>
<th>0.39</th>
<th>0.16</th>
<th>0.48</th>
<th>0.63</th>
<th>0.76</th>
<th>0.69</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.15</td>
<td>0.40</td>
<td>0.68</td>
<td>0.54</td>
<td>0.72</td>
<td>0.62</td>
<td>0.76</td>
<td>0.63</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td>Intra</td>
<td>Inter</td>
<td>Intra</td>
<td>Inter</td>
<td>Intra</td>
<td>Inter</td>
<td>Intra</td>
<td>Inter</td>
<td>Intra</td>
</tr>
<tr>
<td>All cases</td>
<td>Pl -</td>
<td>Pl +</td>
<td>Pl -</td>
<td>Pl +</td>
<td>Pl -</td>
<td>Pl +</td>
<td>Pl -</td>
<td>Pl +</td>
<td>Pl -</td>
</tr>
</tbody>
</table>

Table 4.1. Observed and beyond chance agreement of the histological classification of plasma cell leukemia
blind diagnoses was marginally better than with the reference pathologist’s original diagnoses. Agreement beyond chance tended to be best for the classification of PL+ cases and worst for PL? specimens.

When questionable cases were excluded from the analysis, the kappa values increased substantially (Table 4.2). Compared to the reference pathologist’s blind evaluations, the inter-pathologist agreement on positive versus negative cases approached excellence (k=0.74) when questionables were excluded. Similarly, the intra-pathologist kappa for distinguishing PL+ from PL- reached an excellent level (k=0.81). However, when pathologists B, C and D were compared to A’s original diagnoses, excluding questionable cases, the mean observed and beyond chance agreement remained low (0.69 and 0.28 respectively) despite the increased proportion of PL+ cases in the sample.

Pathologists B, C and D classified more cases as questionable and fewer as positive than did A. The reference pathologist blindly diagnosed more questionable and positive cases, and fewer negative cases than he originally diagnosed. When the frequency of inter-pathologist misclassification was determined there was little difference between the three diagnostic categories. Approximately 64% of the reference pathologist’s original positive diagnoses were classified as negative or questionable by the other pathologists, while 60% of the
<table>
<thead>
<tr>
<th></th>
<th>0.11</th>
<th>0.26</th>
<th>0.81</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.23</td>
<td>0.30</td>
<td>0.74</td>
<td></td>
</tr>
<tr>
<td>0.03</td>
<td>0.06</td>
<td>0.28</td>
<td></td>
</tr>
</tbody>
</table>

Inter-pathologist agreement
(against blind diagnosis)

Inter-pathologist agreement
(against original diagnosis)

(PL+ excluded)
(PL- excluded)

(PL+ versus PL-)
(PL+ versus PL?)

Plasmacytoid Leukemia

Table 4.2, Kappa values for the mean inter- and intra-pathologist diagnoses of...
questionables and 54% of the negative samples were misclassified. When the intra-pathologist comparisons were reviewed, a different pattern emerged. Relative to his original diagnoses, pathologist A misclassified 28% of positives, 48% of negatives and 67% of questionable cases during his blind evaluation of the tests series.

Potential reasons for misclassification were generated by reviewing the comments provided by the participants. Of 200 possible paired diagnoses (4 pathologists x 50 samples), 55 pairs were diagnosed with chronic inflammatory diseases. Forty-five percent of these pairs were concordant (25/55) while 55% (35/55) were discordant with respect to plasmacytoid leukemia classification. Frequently, the pathologists cited difficulties in distinguishing inflammatory reactions from hyperplastic or neoplastic accumulations of cells in the discordant pairs as a source of diagnostic confusion. Insufficient organ selection or tissue autolysis were listed as reasons for diagnosing PL or PL- in only 3% of the 200 pairs; all of these cases came from one pathologist (B).

Because of the consistent trend of B, C and D diagnosing more questionable and less positive cases than the reference pathologist, agreement between B, C and D was calculated to examine if the variation observed was due primarily to disagreements with the reference pathologist. Table 4.3 shows the mean observed and beyond chance
Table 4.3. Mean inter-pathologist agreement on the histological diagnosis of positive (PL+), negative (PL-), and questionable (PL?) cases of plasmacytoid leukemia for pathologists B, C, and D.

<table>
<thead>
<tr>
<th></th>
<th>PL+</th>
<th>PL-</th>
<th>PL?</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observed agreement</td>
<td>0.75</td>
<td>0.66</td>
<td>0.60</td>
<td>0.50</td>
</tr>
<tr>
<td>Kappa</td>
<td>0.35</td>
<td>0.25</td>
<td>0.19</td>
<td>0.36</td>
</tr>
</tbody>
</table>
agreement between B, C and D. These values were judged not to be significantly different from those calculated for the comparison of B, C and D with the blind diagnoses provided by A, but were better than those generated from comparisons made with A’s original diagnoses.

The potential influence of past experience in diagnosing plasmacytoid leukemia on agreement was examined by comparing the individual kappa values of B, C and D, and by review of the intra-pathologist agreement. Relative to A’s original classifications, pathologist B had the highest level of agreement (k=0.36), followed by C (k=0.12), then D (k=-0.03), thus following the anticipated gradient of experience with the disease. However, when compared to the reference pathologist’s blind diagnoses, the kappa values of the other participants were equivalent (B=0.29, C=0.21, D=0.26). These low kappa values, coupled with the poor overall intra-pathologist agreement argue against a substantial influence of previous experience with the disease on the repeatability of histologic diagnosis.

**Discussion**

When a definitive means to determine true disease status does not exist, evaluating the agreement between diagnosticians or tests provides guidance as to the most probable diagnosis. High agreement on a diagnosis is supporting evidence of the validity of the diagnostic
criteria. If there is disagreement, in the absence of knowing which diagnosis is correct, neither the diagnostician's opinion nor the test results are of much value (Martin and Bonnett, 1987). The kappa statistic has become a frequently used measure of the reproducibility and validity of diagnostic procedures (MacClure et al., 1987). Although the relative strength of a specific value of kappa as a measure of agreement has not been definitively established, several guidelines for interpreting this statistic have been published (Martin and Bonnett, 1987; Fleiss, 1981; Landis and Koch, 1977). The kappa values calculated in this study indicate poor agreement for the histologic diagnosis of plasmacytoid leukemia. These findings limit the value of diagnostic records obtained from different fish pathology laboratories as sources of consistent and accurate epidemiologic information about the disease. To improve our ability to study plasmacytoid leukemia, it is important to explore reasons for diagnostic disagreement in order to develop more consistent methods for diagnosing the disease.

The reference pathologist in this study had access to more information for his original diagnosis compared to his blind evaluations. Access to historical information regarding the species involved, the clinical and gross pathological features of some of the cases and the disease status of other fish in the source population had the
potential to substantially influence the classification of
diagnostic samples. Species and concurrent disease have
been seen to affect the diagnosis of plasmacytoid leukemia
(Kent, 1992; Newbound et al., 1991b). Historical information
likely plays a significant role in determining the pre-test
probability for the disease prior to histological
examination. As pre-test probability has a direct influence
on the post-test probability for a diagnosis (Sackett et
al., 1991), historical information likely influences the
histological classification of suspect salmon.

It is unclear if the reference pathologist's original
diagnoses were more or less accurate than his blind
classifications. Classically, blind evaluations have been
considered to provide a better reflection of the true
disease status of a subject when interpreting the results
of a single test (Sackett et al., 1991; Martin and Bonnett,
1987). While some authors contend that the classification
of neoplasia is based primarily on defined histological
criteria (Misdorf, 1990), others claim that the diagnosis
of a specific neoplasm requires an accurate clinical
history (Thomson, 1978). This is particularly the case for
hemic neoplasia where the clinical course can play an
important role in establishing the final diagnosis (Gross,
1983). In this study, the blind diagnoses of the reference
pathologist were in better agreement with the other
participants than were his original diagnoses. This may be
a reflection of the effect of historical information on the histologic diagnosis of the disease. However, as plasmacytoid leukemia is only a recently described disease (Kent et al, 1990), diagnostic experience accumulated between the first and second evaluation of the samples in this study may have altered the diagnostic criteria used by the reference pathologist. Therefore, the low intra-pathologist agreement may, in part, be a reflection of a shift in the criteria for diagnosing marine anemia used by the reference pathologist. In the absence of an independent means to diagnose the true disease status of individual cases, it cannot be determined if the lack of historical data led to the overdiagnosis or underdiagnosis of plasmacytoid leukemia.

Diagnostic consistency can be compromised by the failure to apply standard case definitions, the use of subjective or overlapping diagnostic criteria, unequal diagnostic experience, or poor quality diagnostic materials (Martin and Bonnett, 1987; Stenkvist et al, 1983; Thomas et al, 1983). Previous experience in diagnosing this disease did not consistently bias the blinded classification of samples in this study. Similarly, the quality of diagnostic material was rarely cited as an obstacle to the classification of cases. However, the subjective criteria for dealing with the potential overlap of diagnostic categories was apparently a major source of observer
variation. When the full spectrum of lesions was considered, there was poor agreement on the classification of all the categories of plasmacytoid leukemia created for this study.

The difference between neoplasms, especially hemic neoplasms, and hyperplasia or inflammation can, at times, be subtle (Misdorp, 1990). Problems in making this decision were frequently cited as a source of uncertainty and, therefore, a reason for classifying cases as questionable. The presence of mixed inflammatory reactions, low levels of mitosis, or necrosis and bacteria complicated this differentiation. However, disagreement was not limited to questionable cases. The poor agreement on negative samples emphasizes the difficulties participating pathologists had in differentiating plasmacytoid leukemia from normal samples and from cases with other diseases.

The misclassification of neoplasms because of the obscuring effects of concurrent pathological processes is not unique to plasmacytoid leukemia. Inflammation, necrosis and hemorrhage are features of a variety of neoplasms and can complicate the identification of the primary neoplastic process (Thomson, 1978). Tumours such as mastocytomas, histiocytomas or necrotic sarcomas are at times difficult to differentiate from inflammatory lesions, especially if secondary ulceration or infection has occurred (Misdorp, 1990). Conversely, chronic inflammation, such as in
Aleutian mink disease, can be misdiagnosed as a neoplasm (Jones and Hunt, 1983). An improved understanding of the role of inflammation in the biology of plasmacytoid leukemia would help to clarify the case definition for this disease.

The low reproducibility of histologic classification of neoplasms resulting from variation in the diagnostic criteria used by pathologists has been documented for a number of diseases (Thomas et al, 1983; Stenkvist et al, 1983; Lambourne and Lederer, 1973). In this study, participating pathologists were asked to determine if the samples provided were consistent with a previously published description of the histologic features of plasmacytoid leukemia. This allowed for the individuals to apply their clinical judgement in deciding what constituted sufficient evidence for diagnosing plasmacytoid leukemia. Other descriptions of the disease suggest that the minimum requirements for positive diagnosis are the proliferation of cells resembling plasmablasts in the renal interstitium and at least one non-hematopoietic organ (Kent, 1992). The description provided to pathologists in this study did not specify minimum diagnostic criteria. The lack of a precise case definition in this study was a source of diagnostic disagreement. For example, some pathologists classified cases exhibiting only renal and splenic lesions as positive for the disease, whereas others felt this range of lesions
was inconsistent with a positive diagnosis.

There are opposing views as to how to improve the diagnostic agreement of histopathologists. According to Langley (1978), the most important method of improving the diagnostic accuracy of histopathology is to develop an optimum or set of optimum criteria for diagnosis which employs a number of independent factors in the decision rule. Alternatively, Wilson and Burke (1957), state that "Pathologists should not be forced to agreement with respect to standards of classification in a study due to the danger of modifying their individual standards. One can develop an artificial sense of security by forcing agreement on individuals by strictly applying diagnostic criteria [on subjective procedures such as histology]."

When evaluating a diagnostic method, the potential decrease in agreement due to the failure to provide a strict standard for disease classification must be balanced with the desire to maintain a diagnostic atmosphere similar to that encountered in a regular clinical setting. Although the ideal conditions for histopathologic diagnosis were not created for this study, neither are they always present in regular clinical settings. Despite the lack of published criteria or definitive diagnostic tests for marine anemia, a review of submissions to fish pathology laboratories demonstrated that plasmacytoid leukemia has frequently been diagnosed (Chapter 2). These diagnoses were often made
despite a lack of clinical and historical information. Therefore, although not ideal, the diagnostic setting for this study was not sufficiently different from those encountered in diagnostic laboratories to invalidate these results.

One principle of field investigations of disease is that, from the outset of the investigation, a case definition must be adopted and consistently applied (Last and Sartwell, 1980). Although plasmacytoid leukemia has received recent attention and has been diagnosed in many fish, the results of this study suggest that the disease has not been consistently diagnosed. Efforts to improve the level of agreement for the histopathologic diagnosis of plasmacytoid leukemia are limited by the spectrum of lesions seen in naturally occurring cases and by the diagnostic criteria selected (Kraemer, 1979). The standardization of a case definition for plasmacytoid leukemia would undoubtably improve the agreement of pathologists diagnosing this disease; however, biases and restrictions imposed by the application of exact diagnostic criteria should be identified and acknowledged in future studies of the disease. An alternative to the development of strict histologic criteria for diagnosing plasmacytoid leukemia is the development of a multivariate "decision-tree". The inclusion of historical, microbiological, clinical, and pathological features of cases in the
determination of diagnostic likelihood may provide a more consistent means of correctly categorizing the disease status of a salmon than histological means alone.
References


Appendix 4.1. Proportional intra and inter-pathologist agreement for the diagnosis of positive (PL+), negative (PL-), and questionable cases (PL?) of plasmacytoid leukemia.

### a. Intra-pathologist agreement

<table>
<thead>
<tr>
<th></th>
<th>PL+</th>
<th>PL-</th>
<th>PL?</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>PL+</td>
<td>0.27</td>
<td>0.10</td>
<td>0.05</td>
<td>0.42</td>
</tr>
<tr>
<td>PL-</td>
<td>0</td>
<td>0.23</td>
<td>0.08</td>
<td>0.31</td>
</tr>
<tr>
<td>PL?</td>
<td>0.10</td>
<td>0.10</td>
<td>0.07</td>
<td>0.27</td>
</tr>
<tr>
<td>Total</td>
<td>0.37</td>
<td>0.43</td>
<td>0.20</td>
<td>100%</td>
</tr>
</tbody>
</table>

### b. Mean inter-pathologist agreement (versus reference pathologists original diagnoses)

<table>
<thead>
<tr>
<th></th>
<th>PL+</th>
<th>PL-</th>
<th>PL?</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>PL+</td>
<td>0.14</td>
<td>0.08</td>
<td>0.05</td>
<td>0.26</td>
</tr>
<tr>
<td>PL-</td>
<td>0.07</td>
<td>0.19</td>
<td>0.08</td>
<td>0.34</td>
</tr>
<tr>
<td>PL?</td>
<td>0.18</td>
<td>0.15</td>
<td>0.07</td>
<td>0.40</td>
</tr>
<tr>
<td>Total</td>
<td>0.38</td>
<td>0.42</td>
<td>0.20</td>
<td>100%</td>
</tr>
</tbody>
</table>

### c. Mean inter-pathologist agreement (versus reference pathologists blind diagnoses)

<table>
<thead>
<tr>
<th></th>
<th>PL+</th>
<th>PL-</th>
<th>PL?</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>PL+</td>
<td>0.19</td>
<td>0.04</td>
<td>0.03</td>
<td>0.26</td>
</tr>
<tr>
<td>PL-</td>
<td>0.07</td>
<td>0.17</td>
<td>0.08</td>
<td>0.34</td>
</tr>
<tr>
<td>PL?</td>
<td>0.16</td>
<td>0.10</td>
<td>0.14</td>
<td>0.40</td>
</tr>
<tr>
<td>Total</td>
<td>0.42</td>
<td>0.31</td>
<td>0.27</td>
<td>100%</td>
</tr>
</tbody>
</table>
CHAPTER 5

MARINE ANEMIA IN FARmed CHINOOK SALMON (ONCORYHNCHUS TSHAWYTSCHA): DEVELOPMENT OF A WORKING CASE DEFINITION

Introduction

Marine anemia, also known as plasmacytoid leukemia, is a recently described disease of farmed chinook salmon. The disease is characterized by the massive proliferation of immature lymphoblasts resembling plasma cells, in a wide variety of tissues (Kent et al., 1990; Kent, 1992). Because the disease has been identified as the apparent cause of epidemic levels of mortality, marine anemia has become a significant cause of concern for salmon farmers and researchers alike (Anon, 1992; Kent et al., 1990; Newbound et al., 1993; Eaton and Kent, 1992). However, controversy exists regarding the distribution, etiology and natural history of the disease on salmon farms (Newbound and Kent, 1991; Brackett et al., 1990).

Perhaps the major factor underlying the controversy about the syndrome has been the lack of a widely applicable case definition. Diagnosing marine anemia using clinical and gross pathological features has, in the past, been considered impossible (Kent, 1992). For this reason, many
of the published descriptions of the disease have relied almost exclusively on histological features to establish a diagnosis of marine anemia (Kent, 1993; Newbound et al., 1993; Kent and Dawe, 1993). The dependence on histologic criteria has limited the ability of investigators to comprehensively study the natural history of the disease on salmon farms. The relatively rapid autolysis of dead salmon in seapens (Kent, 1992) has deterred researchers from using mortalities when investigating the affects of marine anemia on the mortality pattern of affected salmon farms. In addition, significant disagreement regarding the marine anemia status of a fish can occur when histologic evaluation is the sole criteria used for diagnosis (Chapter 4). The lack of a standardized diagnostic criteria has been implicated as an important source of such disagreement (Chapter 4).

The purpose of obtaining a diagnosis is to correctly classify the disease status of a patient or population so that, based on past experience, appropriate recommendations can be made or actions taken to deal with the diagnosed problem (Sackett et al., 1991, p. 4). In epidemiologic studies, the ideal diagnostic criteria used should be capable of identifying all members of the population that have a high probability of being affected by the disease and capable of excluding the majority that do not (Last and Sartwell, 1980). The true disease status of a patient can
rarely be directly observed and must therefore be inferred from imperfect clues such as history, physical examination and diagnostic tests (Sox et al, 1988). Representing uncertainty about a diagnosis as a probability is an important first step in making a medical decision (Sox et al, 1988). A variety of multivariate statistical techniques have been employed to quantify diagnostic probability (Diehr et al, 1981; Hanley and McNeil, 1982; Hanley, 1983). Diagnostic models developed by these techniques allow one to attempt to achieve optimal discrimination of the disease status of the patient or population by consistently applying mathematically derived rules. However, the validity of these mathematical decision rules is dependent on the ability of the diagnostician to accurately identify the signs and symptoms of the patient.

The objectives of this study were to identify the salient histological diagnostic features of marine anemia and to devise a method for diagnosing the disease in dead fish. By creating a method capable of reliably identifying the marine anemia status of sacrificed and dead fish in seapens, the scope of future epidemiological and clinical studies of the disease can be expanded.

Materials and Methods
Sources of data

Data were collected from commercial chinook salmon
farms in British Columbia, Canada between 1991 and 1993. An initial case series was collected from a complete list of approximately 200 cases of marine anemia at a federal fish health laboratory (Department of Fisheries and Oceans, Nanaimo, B.C.) from 1987-1992, using a random number table. All cases had previously been diagnosed by a fish pathologist with extensive experience with clinical and experimental cases of the disease. Ninety routinely prepared, hematoxylin and eosin stained tissue sections were available for light microscopic examination (Humanson, 1979). This series was used to describe the common histologic features of cases previously diagnosed as positive for marine anemia. At least seven of the eight following organs were present in all of the tissue samples: caudal kidney, liver, spleen, colon, heart, pyloric cecae, mesenteric fat, and pancreas.

Development and evaluation of histologic criteria

As previous descriptions of fish with marine anemia describe the proliferation of immature lymphoblasts as a key diagnostic feature (Kent et al, 1990), the occurrence of such proliferations were described and quantified in the organs selected for this study. To describe changes in hematopoietic cells, the degree of renal interstitial hyperplasia was estimated by calculating the average number of cross-sections of renal tubules present per three 40X
light microscopic field of sections of the caudal kidney. To account for the greater area occupied by longitudinal sections of renal tubules, a "tubule score" was created as an empirical means to quantify changes in the renal interstitium. This score equalled the sum of each tubule cross-section plus three times the sum of longitudinal sections. Differential cell counts were conducted on the interstitium of the caudal kidney in order to further describe hematopoietic activity. One hundred cells were counted for each case. Cells were classified as mature red blood cells, polymorphonuclear cells, small mononuclear cells, medium-sized mononuclear cells, large mononuclear cells, blast cells or mitotic figures. Mean tubule scores and differential cell counts were compared statistically by t-tests (Colton, 1974, pp.127-130).

Features consistently identified in positive cases in the retrospective series were used to develop a histological diagnostic criteria. The validity and repeatability of the new criteria were evaluated by measuring intra-observer diagnostic agreement. Fifty fish were obtained from commercial farms and diagnosed by a single diagnostician as being positive or negative for marine anemia using the new histologic criteria. All questionable cases were classified as negative. One month later, the same series was blindly re-evaluated by the same diagnostician. Agreement was measured by calculating the
observed agreement and the kappa coefficient. Kappa is a measurement of agreement that is often used as a measure of the validity of a diagnostic test when no "gold standard" is available for confirming the diagnosis (MacClure and Willett, 1987). Criteria for interpreting kappa were taken from Fleiss (1981).

Development and evaluation of gross pathologic criteria

In the second portion of the study, 197 salmon were collected from the surface of seapens to identify the gross pathological features that were associated with the histological criteria developed in the first half of the study. Fish were captured alive, euthanized by cervical severance, and then subjected to clinical and gross pathological examinations. Fish were classified as positive or negative for marine anemia after reviewing routinely prepared, hematoxylin and eosin stained tissue sections. The same tissues were available for review for each fish examined. The numbers of fish demonstrating specific clinical and gross pathological lesions were tabulated. The association of each symptom with a positive histological diagnosis was tested using a chi-squared test (Colton, 1974, pp. 175-179). Odds ratios were calculated to estimate the magnitude of these associations (Martin et al, 1987, pp. 130-133).

A diagnostic algorithm was constructed to discriminate
cases from non-cases of marine anemia using gross pathological and clinical signs alone. In the process of developing the algorithm, a series of "yes-no" questions regarding the presence of a specific lesion was answered. The lesion that best distinguished the two diagnostic groups as indicated by the most significant chi-squared value was selected as the lesion best able to determine a salmon’s marine anemia status. Each of the two sub-groups that resulted from the "yes-no" decision node was then subjected to a second "yes-no" question concerning the presence of a different lesion, again, selecting the best lesion for diagnostic differentiation by statistical criteria. The process was continued until positive and negative fish could no longer be further discriminated. The probability of marine anemia diagnosis for each branch of the algorithm was calculated by applying Bayes formula (Eddy and Clanton, 1982). Branches of the algorithm for which the probability of positive diagnosis equaled or exceeded 50% were classified as diagnostic for the disease, while those with less than a 50% probability were considered branches negative for marine anemia.

To assess the ability of the algorithm to discriminate marine anemia from non-marine anemia fish, diagnoses based on the algorithm were compared to those determined by the histological criteria developed for the study. The overall correct classification rate was defined as the proportion
of salmon with algorithm and histological diagnosis in agreement. The sensitivity of the model was determined by calculating the proportion of histologically positive fish classified as positive by the algorithm, while the specificity was defined as the proportion of histologically negative fish correctly classified by the algorithm.

The performance of the algorithm was also evaluated at the group level. By applying the formulas provided in Martin et al. (1992), the probability of detecting disease in disease positive groups (GSENS or Group SENSitivity) and the probability of not detecting disease in a disease free group (GSPEC or Group SPECificity) were tabulated for various levels of disease prevalence and numbers of fish sampled per group.

Results

Table 5.1 documents the frequency of specific organ involvement in previously diagnosed cases of marine anemia. The two hematopoetic tissues, the kidney and the spleen, were the most frequently involved organs. Blast cells could also be found in retrobulbar tissues and the meninges; however, these organs were rarely included in the samples reviewed in the retrospective series. All cases had involvement of organs other than the spleen and kidney. Blast cells were found in 5 or more tissues in 39% of the cases, in 4 tissues in 15%, in 3 tissues in 22%, and in
Table 5.1. Distribution of lesions in specific organs in positive cases of marine anemia

<table>
<thead>
<tr>
<th>ORGAN</th>
<th>PERCENTAGE OF CASES WITH SPECIFIC ORGAN INVOLVEMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>99%</td>
</tr>
<tr>
<td>Spleen</td>
<td>89%</td>
</tr>
<tr>
<td>Heart</td>
<td>49%</td>
</tr>
<tr>
<td>Liver</td>
<td>47%</td>
</tr>
<tr>
<td>Colon</td>
<td>44%</td>
</tr>
<tr>
<td>Pyloric Cecae</td>
<td>29%</td>
</tr>
<tr>
<td>Pancreas</td>
<td>17%</td>
</tr>
</tbody>
</table>
only 2 tissues in 24% (mean = 4 tissues/case).

Renal interstitial hyperplasia was a common feature of all positive cases (Figure 5.1). Sections of the caudal kidney deemed to be hyperplastic had, on average, approximately 50% of the number of tubule cross-sections compared to samples identified as normal renal tissue (p < 0.01). The hyperplasia in marine anemia positive cases was characterized by an increase in the proportion of large mononuclear cells, blast cells and mitotic figures. These cells accounted for a mean value of 22% of the renal interstitial cells in positive case as compared to only 8% in healthy farmed salmon (p < 0.01). Although hyperplasia and differential cell count could distinguish cases of marine anemia from healthy fish, these characteristics were insufficient to rule out all non-cases.

To further distinguish marine anemia, organs other than the kidney needed to be examined. All positive cases had mononuclear cell infiltrates in at least one organ other than the spleen and kidney. These infiltrates were composed predominantly of large mononuclear cells and blast cells. It was not possible to classify these cells as plasma cell precursors on light microscopic examination. The presence of obvious sources of chronic inflammation such as necrosis or granuloma formation usually precluded the diagnosis of marine anemia; however, the presence of micro-organisms such as *Renibacterium salmoninarum* or
Figure 5.1: Mean tubule score and proportion of large cells in the renal interstitium of apparently healthy fish and fish with marine anemia (bars = mean; lines = standard deviation).
Enterocytozoon salmonis, did not preclude such a diagnosis.

Histological features that were identified in the majority of previously diagnosed cases of marine anemia were used as the basis for a working case definition. To be considered a positive case, the following characteristics had to be present: (1) hyperplasia of the interstitial cells of the caudal kidney characterized by a "tubule score" of less than 25; (2) an increased proportion (> 15%) of large mononuclear cells, blast cells and mitotic figures in the caudal renal interstitium; (3) the presence of mononuclear cell infiltrates, composed primarily of large mononuclear cells and blast cells in at least one organ other than the spleen or kidney; and (4) no significant signs of granuloma formation or necrosis in the organs examined. Conservative parameters were used for the tubule score and proportion of large mononuclear cells in the renal interstitium in order to not restrict the sensitivity of the criteria. These features were tested as a criteria for diagnosis by conducting an intra-observer agreement trial. The observed agreement was 0.94 and the kappa coefficient was 0.84. This kappa value is indicative of excellent agreement and thus allowed for the confident use of the criteria for classifying cases. Reliance on these criteria for the diagnosis of marine anemia excluded some cases that had been previously classified as positive for
the disease. The latter cases either failed to involve organs other than the kidney and spleen, revealed blast cells in retrobulbar tissue only, or had significant signs of tissue necrosis or granuloma formation.

Of the 197 salmon collected from seapens that were subjected to the above diagnostic criteria, 39 (20\%) were classified as positive. Most of the gross lesions observed in this prospective case series fell into one of three general categories: (1) enlargement or thickening of visceral organs, specifically the kidney, spleen, liver and lower intestine; (2) changes in the colour of organs, such as pallor of the gills or visceral tissues, and darkening of the spleen; and (3) gross signs of inflammatory processes such as granuloma formation, fibrin production or excessive petechiation (Table 5.2). Although the proportion of salmon with renomegaly, splenomegaly, and hepatomegaly were significantly different in marine anemia positive and negative groups, no single lesion was able to adequately discriminate the two groups of salmon. Of particular interest was the infrequency of gill pallor in positive cases, despite the common name for the disease.

Although the prevalence of six of the nine recorded gross lesions were not significantly different in marine anemia positive and negative fish, all categories but gill pallor and organ discoloration were useful in developing the diagnostic algorithm. Only five gross observations were
<table>
<thead>
<tr>
<th></th>
<th>ANEMIA CASES</th>
<th>CASES</th>
<th>MARINE ANEMIA</th>
<th>ADJ.RATIO</th>
<th>P-VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.40</td>
<td>0.10</td>
<td>22%</td>
<td>10%</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>0.21</td>
<td>0.20</td>
<td>5%</td>
<td>10%</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>0.29</td>
<td>0.16</td>
<td>8%</td>
<td>15%</td>
<td>0.28</td>
<td></td>
</tr>
<tr>
<td>0.29</td>
<td>0.29</td>
<td>28%</td>
<td>20%</td>
<td>0.29</td>
<td></td>
</tr>
<tr>
<td>1.77</td>
<td>0.13</td>
<td>23%</td>
<td>35%</td>
<td>0.28</td>
<td></td>
</tr>
<tr>
<td>1.47</td>
<td>0.28</td>
<td>31%</td>
<td>40%</td>
<td>0.28</td>
<td></td>
</tr>
<tr>
<td>7.05</td>
<td>&gt;0.001</td>
<td>11%</td>
<td>48%</td>
<td>0.28</td>
<td></td>
</tr>
<tr>
<td>8.13</td>
<td>&gt;0.001</td>
<td>27%</td>
<td>75%</td>
<td>0.28</td>
<td></td>
</tr>
<tr>
<td>13.77</td>
<td>&gt;0.001</td>
<td>40%</td>
<td>90%</td>
<td>0.28</td>
<td></td>
</tr>
</tbody>
</table>

Derived from chi-squared tests.

Table 5.2: Frequency and significance of gross pathological lesions associated with marine anemia.
required to discriminate the two groups of salmon. These observations included the presence or absence of, renomegaly, splenomegaly, hepatomegaly, "other marine anemia lesions", and gross signs of inflammation or infection.

Fish were considered positive for the category "other marine anemia lesions" if ascites, colonomegaly or exophthalmia, each a sign previously associated with the disease (Kent, 1992), were present. By combining these five gross features, an algorithm could be developed that was capable of classifying 87% of the salmon the same as they had been histologically classified (Figure 5.2). The model was better at classifying marine anemia negative fish (91% specificity) than positive fish (71% sensitivity). The final algorithm provided two branches for positive diagnosis (probability >50%) and six branches for negative diagnosis (probability <50%).

Table 5.3 reveals the performance of the algorithm when used to establish the marine anemia status of a group of salmon. For this table, the detection of only one positive fish was considered sufficient to classify a group as positive. At low prevalence, relatively large samples per group were required to obtain a reasonable probability of correctly classifying a group as positive for marine anemia. In groups affected by a high prevalence of the disease (>30%), relatively few fish would be needed to
Figure 5.2: Algorithm for diagnosis of marine anemia in farmed salmon using gross pathological and clinical findings (p = probability of positive diagnosis)
Table 5.3. Group-level sensitivity (GSENS) and specificity (GSPEC) for different levels of marine anemia prevalence (MA PREV) and number of salmon sampled per pen. The criteria for declaring a pen positive was one positive fish per group.

<table>
<thead>
<tr>
<th>GSENS:</th>
<th>NUMBER OF FISH TESTED</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>MA PREV</td>
<td></td>
</tr>
<tr>
<td>0.01</td>
<td>0.19</td>
</tr>
<tr>
<td>0.05</td>
<td>0.23</td>
</tr>
<tr>
<td>0.10</td>
<td>0.28</td>
</tr>
<tr>
<td>0.20</td>
<td>0.38</td>
</tr>
<tr>
<td>0.30</td>
<td>0.48</td>
</tr>
<tr>
<td>0.50</td>
<td>0.64</td>
</tr>
<tr>
<td>0.75</td>
<td>0.80</td>
</tr>
<tr>
<td>1.00</td>
<td>0.92</td>
</tr>
</tbody>
</table>

GSPEC:

|        | 0.00 | 0.00 | 0.00 | 0.00 |

|        | 0.82 | 0.62 | 0.39 | 0.15 |
correctly classify the diseased pen when using the algorithm for diagnosis. However, the probability of correctly diagnosing a disease-free group as negative rapidly decreased as the sample size increased.

Discussion

The objective of epidemiological studies is to accurately measure the frequency, distribution, and determinants of health and disease in populations, with the ultimate goal of decreasing the incidence and impact of the disease (Martin et al., 1987, p. 3; Rothman, 1986). To achieve this goal one requires a clear, explicit case definition and valid methods of measuring the disease in the population (Rothman, 1986). The lack of a standard case definition for marine anemia has restricted the breadth and accuracy of past epidemiologic studies of the disease (Chapter 4). This study presents an internally valid case definition for a specific histologically defined syndrome that is consistent with previous descriptions of marine anemia (Kent et al., 1990, Kent and Dawe, 1993). In addition, a criterion capable of reliably classifying the marine anemia status of a fish on the basis of gross pathological signs alone was developed.

When the spectrum and combinations of gross signs of marine anemia were incorporated into an arborizing strategy of diagnosis, a salmon's marine anemia status could be
determined with a reasonable level of certainty. The algorithm presented in this report can be used to directly measure the occurrence of marine anemia in dead salmon, thus eliminating the need to rely on assumptions regarding the correlation of disease patterns observed in live-caught fish with the pattern of fatal diseases in a seapen. Positive results for a diagnostic test with high specificity are sufficient evidence for ruling in a target disorder (Sackett et al, 1991, p. 83). Therefore, despite the moderate sensitivity of the algorithm, the high specificity allows the model to act as a good diagnostic tool for ruling in individual cases of marine anemia.

When using the diagnostic algorithm to categorize the marine anemia status of a group of salmon, the performance of the algorithm is affected not only by its sensitivity and specificity, but also by the prevalence of the disease in the population and the number of fish sampled (Martin et al, 1992). Previous studies have suggested that the prevalence of marine anemia on salmon farms in British Columbia can range from 20%-50% (Brackett et al, 1991; Kent et al, 1990). Within this range of prevalence, the examination of 10 fish per group will almost certainly result in the correct classification of positive groups. However, because the group specificity declines with increasing sample size, samples of 10 fish or more have a high probability of classifying a group as positive,
regardless of its true disease status. Because of the moderate sensitivity of gross pathologic diagnosis of marine anemia in individual fish, and the rapidly declining group level specificity with increasing sample size, definitive diagnosis of the disease still requires histology.

Superficially, the procedures used to develop the case definition presented in this report may appear to be an example of circular reasoning. A series of cases was collected using a specific set of diagnostic criteria in order to develop a definition for the disease. However, the purpose of this study was not to redefine marine anemia but was instead to identify the subset of lesions that would allow for maximal discrimination of cases from non-cases. Although the working definition attempts to standardize the diagnosis of marine anemia, several factors may restrict the application of this criterion. The definition does not allow for the identification of all cases of marine anemia. As the signs and symptoms included in the working definition were observed in surface moribund and dead salmon, this definition of marine anemia is unlikely to detect early, subclinical, or recovered cases. Early markers for the disease or a means to identify an etiologic agent will be required before the relationship of marine anemia to seafarm morbidity and production can be studied. Therefore, the working cases definition developed in this
study should be restricted to mortality studies. However, as mortality, and not sub-lethal effects, are a primary concern of the British Columbia salmon farming industry (Anon, 1992), this definition has clinical applications.

The inter-observer agreement of the diagnostic criteria presented above has not been determined. Historically, inter-pathologist disagreement has been a concern for the histologic diagnosis of marine anemia (Chapter 4). As the lack of a standard criteria for diagnosing marine anemia has been cited as a potentially important reason for inter-pathologist disagreement (Chapter 4), strict application of this working definition may improve the reliability and repeatability of histologic diagnosis. However, because of the historical problems with inter-observer variation, when applying the case definition described above, it is best to have a single observer make all of the diagnoses, and to assess this persons diagnostic variation (Last, 1986).

Although this study provides a repeatable, consistent set of histological criteria for use in the study of marine anemia, it has not identified the pathognomic lesions of a specific disease. The pathologic implications of the histological lesions classically associated with marine anemia has been a source of some debate. Difficulties distinguishing chronic inflammation from neoplasia in salmon have been a major stumbling block in classifying a
fish’s marine anemia status and has been a significant cause of diagnostic disagreement (Chapter 4). Difficulties in differentiating inflammation and neoplasia are not restricted to marine anemia. Aleutian disease of mink (Jones and Hunt, 1983), sporozoan associated lymphomas in turbot (Ferguson and Roberts, 1975) and chronic lymphoblastic leukemia of man (Rosenblatt et al., 1991), are examples of diseases subject to similar diagnostic debates. In addition, histologically similar cases need not share the same etiologies. Although squamous cell carcinomas of the lung of man are virtually always associated with cigarette smoking, other causal factors have also been implicated (Tisi, 1980). Similarly, although a retrovirus has been causally associated with marine anemia (Eaton and Kent, 1993), one cannot conclude that all fish exhibiting histologic lesions consistent with marine anemia are the result of retroviral infections. Although this study does not resolve the debates regarding the pathology and etiology of marine anemia, it does provide investigators with a reliable case definition that can be more confidently applied to future research. The more explicit definition presented in this study allows one to consistently identify a specific histologically defined syndrome and thus increases the likelihood of future research detecting significant associations, if such associations exist (Holmes et al., 1988).
References


diagnosis: Solving the clinicopathological exercise.

associated with sporozoan infection in cultured turbot
(Scophthalmus maximus L.). J. Comp. Path., 85: 317-
326.

Proportions, 2nd edn. John Wiley and Sons, Toronto:
pp. 212-235.


discrimination and utilization of radiologic

12. Holmes, G.F., Kaplan, J.E., Gantz, N.M., Komroff, A.L.,
Schoberger, L.B., Strauss, S.E., Jones, J.F., Dubois,
R.E., Cunningham-Rundles, C., Pahwa, S., Tosato, G.,
Zegans, L.S., Purtilo, D.T., Brown, N., Schooley,
R.T., and Brus, I., 1988. Chronic fatigue syndrome: A


CHAPTER 6
MORTALITY SURVEYS AS A TOOL FOR STUDYING MARINE ANEMIA IN SEAPEN REARED SALMON

Introduction

Fatal diseases are a major factor limiting the economic viability of the salmon farming industry in British Columbia, Canada. In 1992, estimates of the direct losses from disease ranged from 15-30% of fish prior to harvest, an economic loss of over $20-$30 million annually (Anon, 1992). These losses have led British Columbia salmon farmers to identify as a primary industry objective research that is designed to develop effective methods of disease prevention and control (Anon, 1992). However, before a rational disease control and prevention program can be developed, reliable information about the extent and pattern of the disease problem is needed (Elliott and Tattersfield, 1979).

Representative selection of cases, and complete detection or reporting of disease are essential for unbiased descriptions of the extent of a disease in a defined population (Martin et al, 1987 p. 262). To quantify
the extent of a fatal disease, it is generally necessary to examine the mortalities. However, the examination of mortalities is not commonly exploited as a tool for disease investigation on fish farms. The reliance on microbiological and microscopic features for diagnosing many salmon diseases (Kent, 1992; Pillay, 1990) has encouraged fish health investigators to focus their diagnostic efforts primarily on live fish (Pettijohn, 1983; McDaniel, 1979). Because of the rapid autolysis and post mortem contamination of dead fish (Kent, 1992), the examination of mortalities in seapens has been considered of limited value when investigating diseases of farmed salmon (Brackett et al, 1990; Kent, 1992).

Experience in other animal production industries suggests that useful information can be gained from performing routine gross post-mortem examinations of mortalities. Recent work in the cattle feedlot industry, using necropsy surveys with minimal microbiological and microscopic involvement, has led to a better understanding of the prevalence and natural history of a number of important feedlot diseases (Kelly, 1984; Ribble et al, 1991; Ribble, 1992). Mortality surveys have also generated insights into the pattern of mortality in some wildlife species, despite the pervasive problems of carcass autolysis and contamination (Stephen and Burger, [in press]).
Marine anemia is a recently described disease of farmed chinook salmon in British Columbia. The disease, also known as plasmacytoid leukemia, has been associated with mortality rates estimated to be as high 50% (Kent et al., 1990). Marine anemia is characterized by the invasion and proliferation of blast cells, resembling immature plasma cells, into a number of visceral tissues (Kent et al., 1990). The perception that marine anemia can be the cause of devastating mortality rates developed despite a lack of direct information regarding the frequency of the disease in mortalities. As with many other salmon diseases, it has been assumed that autolysis would prevent the identification of the characteristic histological features of marine anemia in dead fish. As a result, estimates of the impact of marine anemia on mortality rates have generally been extrapolated from samples of moribund fish captured from the surface of the pens (Brackett et al., 1991; Brackett et al., 1990; Newbound and Kent, 1991). However, the disease pattern of surface moribund fish may not represent the general mortality pattern of a seapen (Chapter 3). The current inability to directly quantify marine anemia in dead fish limits attempts to determine the true impact of the disease and to discover factors associated with the occurrence and distribution of marine anemia. The principle objectives of this study were to establish if marine anemia could be diagnosed and
quantified in seapen mortalities, and to identify factors that can increase the value of mortality surveys for studying the epidemiology of the disease.

Materials and Methods

Sources of data

This study was conducted on seven commercial chinook salmon (Oncorhynchus tshawytscha) farms in southern British Columbia, Canada from July, 1992 to December, 1993. The design and management of participating farms were typical of standard seapen aquaculture practices in British Columbia. Fish were housed in 3500m³ or 2500m³ cubical netpens. The ages of fish included in the study ranged from nine-month old smolts, which had been recently introduced to seawater, to four-year old broodstock. Participating farms were selected on the basis of convenient access and owner cooperation. Farm managers were asked to not alter their routine management practices for the purposes of the study, in order to evaluate the mortality surveys under conditions similar to those encountered by fish health practitioners in British Columbia.

A total of 31 farm visits were conducted. During each visit, fish were collected from the surface and from the bottom of four adjacent seapens. Fish captured by dipnets from the surface were alive, but assumed to be moribund. After capture, fish were euthanized by cervical severance
prior to examination. A minimum of two hours per visit was devoted to the collection of surface moribund fish. Dead fish were collected for the mortality surveys by exhaustive searches of the pen bottoms by scuba divers. The time since mortalities were last recovered from each pen was recorded, as were the mortality rates for the week of the visit and the water temperature at 5m depth. Mortality rates for study pens were taken from farm records. Farm managers calculated weekly mortality rates by dividing the number of dead fish recovered from a pen for a week by the estimated number of fish in the pens. Estimates of the total number of fish in the pen were derived from periodic counts of the all fish in the pen and were adjusted over time by subtracting the numbers of dead fish recovered from the pens.

Diagnositic criteria

All fish were subjected to gross post-mortem examinations. The degree of post-mortem change, the colour of the gills and any significant gross pathological lesions were noted on standardized forms at the time of necropsy. Tissue sections were collected from all surface caught fish and a sub-sample of the dead salmon, and stored in Davidson’s solution (Humanson, 1979). Tissues were later routinely prepared for histological examination and stained with hematoxylin and eosin (Humanson, 1979). The degree of
autolysis seen upon histopathological examination was noted for each case, as were any lesions pertaining to the diagnosis of marine anemia.

The marine anemia status of each fish was determined by applying a previously developed case definition for the disease (Chapter 5). This case definition provided histological and gross pathological criteria for the diagnosis of marine anemia. In order for a fish to be amenable to gross pathological diagnosis, the following key features had to be recognizable at gross necropsy: (1) enlargement of visceral organs, particularly the kidney, spleen, liver, and colon, (2) exophthalmia, (3) ascites, and (4) an inflammatory process such as granulomas, fibrin production or excessive petechiation. To obtain a histological diagnosis, it was essential for the histopathologist to determine if the following were present or absent: hyperplasia of the hematopoietic cells in the caudal renal interstitium; the proliferation of large mononuclear cells, blast cells and mitotic figures in the caudal renal interstitium; the presence of infiltrates of mononuclear cells in tissues other than the spleen or kidney; and significant histological signs of inflammation or necrosis. A sample was judged to be good for disease classification when all of the gross and histological features needed for making a diagnosis of marine anemia could be recognized. Fair samples were those that allowed
no more than a subjective estimate that the probability of a positive diagnosis was either above or below 50%, but autolysis prevented definitive diagnosis. Poor samples were those in which post mortem changes prevented making a diagnosis. A farm was considered to be positive for marine anemia if one fish fitting the gross and histopathological criteria established in chapter five was found in any of the four pens examined.

Analysis

Linear regression and Pearson's correlation coefficient ($r$) (Colton, 1974. pp. 189-218) were used to explore the relationship of management and environmental variables with the proportion of dead fish that were found unsuitable for marine anemia diagnosis. The management and environmental variables that were investigated included the interval between collection of mortalities, the water temperature, and the weekly mortality rate. The assumptions of linear regression were addressed by reviewing the data for extreme values, plotting the fitted values of the final model against the standardized residuals, and by insuring independence of the outcome variable. Selection of the final linear model was based on the coefficient of determination ($R^2$) (Draper and Smith, 1981), Mallow's statistic ($C(p)$) (Draper and Smith, 1981), and the statistical significance of the model. The statistical
significance of calculated r values was determined by referring to table values in Colton (1974, p.349). The statistical analysis was conducted on a commercial software package (Statistix 4.0, Analytical Software, St.Paul, Minnesota, U.S.A.).

Results

Over the course of this study, 1061 dead salmon were recovered from the bottom of 124 seapens (Table 6.1). From these same pens, only 152 surface moribund fish could be captured. The ratio of the number of mortalities in a pen to the number of surface moribund fish captured ranged from 1:1 to 20:1. On average, for every seven dead salmon recovered from the bottom of the study pens, only one moribund fish could be caught at the surface. No surface moribund fish could be captured on 10% of the farm visits. The number of fish caught at the surface relative to the numbers of mortalities in a pen was not related to the pen mortality rate (r = 0). However, the frequency of retrieval of dead fish from the bottom of a netpen did positively influence this ratio (r = 0.49, p< 0.05).

Judgements regarding the size of visceral organs, signs of inflammation, and the presence of ascites or exophthalmia could be confidently made in 85% (n=902) of the dead fish that were examined. Fifteen percent (n=159) of the dead fish were considered too severely autolyzed for
Table 6.1. Farm-to-farm variation in the number of surface moribund (SCF) and dead fish accessible for examination.

<table>
<thead>
<tr>
<th>FARM</th>
<th>NUMBER OF SCF’s</th>
<th>NUMBER OF DEAD FISH</th>
<th>NUMBER OF FARM VISITS</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>35</td>
<td>315</td>
<td>8</td>
</tr>
<tr>
<td>B</td>
<td>22</td>
<td>181</td>
<td>8</td>
</tr>
<tr>
<td>C</td>
<td>6</td>
<td>55</td>
<td>1</td>
</tr>
<tr>
<td>D</td>
<td>9</td>
<td>43</td>
<td>2</td>
</tr>
<tr>
<td>E</td>
<td>44</td>
<td>143</td>
<td>6</td>
</tr>
<tr>
<td>F</td>
<td>26</td>
<td>309</td>
<td>2</td>
</tr>
<tr>
<td>G</td>
<td>10</td>
<td>15</td>
<td>4</td>
</tr>
<tr>
<td>TOTAL</td>
<td>152</td>
<td>1061</td>
<td>31</td>
</tr>
</tbody>
</table>
gross pathological examination. Under typical conditions of fish farm management in B.C., mortality surveys provided approximately six times more fish (902 vs 152) for the gross pathological determination of a farm's marine anemia status than did samples of surface moribund fish alone.

When severely autolyzed salmon were excluded from further examination, 35% (n=316) of the remaining mortalities provided good samples for the histological diagnosis of marine anemia, 17% (n=153) provided fair samples and the remaining 48% (n=432) were unsuitable for histological diagnosis. Therefore, of the 1061 dead fish collected from the pen bottoms, 469 (44%) provided good or fair histological information that was helpful in making a final decision regarding a salmon's marine anemia status. Mortality surveys increased the number of fish suitable for histopathological diagnosis of marine anemia by three times more than the number of salmon that could be captured from the surface of seapens under normal management conditions (469 vs 152).

The degree of gill pallor of the carcasses provided a guide for judging the quality of histological samples that could be obtained from a dead fish. Only 19% of the mortalities with white gills provided good histological samples. However, as the degree of pallor decreased, the quality of dead fish for histopathological examination increased. Fish with light pink gills provided good samples
50% of the time, fish with dark pink gills 83% of the time and 100% of the cases with red gills provided samples which were good for evaluating a fish for marine anemia. However, under the conditions of this study, only 18% of the recovered mortalities had red or dark pink gills, while 14% had light pink gills and the remaining 68% of the carcasses had white gills. Not surprisingly, pens in which mortality recovery dives were infrequent had a higher proportion of carcasses with pale gills than pens that were frequently dived. Gill pallor was not, however, an absolute indicator of the potential histological quality of a case. The presence of significant pallor in moribund fish that yielded excellent histological samples precluded the use of gill colour as an indicator of histological quality in moribund salmon. As 35% of the dead fish with white gills provided good samples, other indicators of the degree of decomposition were also taken into consideration. Marked scale loss, corneal opacity and liquifaction of organs, were always associated with histological samples of poor quality.

When applying gross and histopathological criteria for determining the marine anemia status of a farm, there was poor agreement between the results of the mortality surveys and the surface moribund fish samples. When the "true" marine anemia status of a farm was taken to be the results of the mortality survey, the samples of surface moribund
fish correctly classified only 47% of the positive pens and 85% of the negative pens. However, the mortality surveys did not invariably detect marine anemia on positive farms. On 13% of the visits to positive farms, marine anemia could be found in surface moribund fish, but not in the dead fish that were examined.

Linear regression and correlation analysis (Tables 6.2 and 6.3) suggested that the interval between mortality collection dives strongly influenced the proportion of dead fish suitable for marine anemia diagnosis ($R^2=0.67$). When only one day elapsed between mortality dives, the mean proportion of dead fish deemed of no value for marine anemia diagnosis was 5%; for an interval of two days, the mean proportion climbed to 13%; when three or more days passed between mortality dives, the mean proportion of dead fish unsuitable for diagnosis reached 37%. Including water temperature and weekly mortality rate in the linear model did not explain a significant additional amount of farm to farm variation in the proportion of dead fish unsuitable for making a diagnosis of marine anemia. There were significant correlations between the independent variables offered to the regression model. However, neither water temperature nor weekly mortality rate was capable of explaining the variation in the proportion of dead fish
Table 6.2. Factors associated with the proportion of dead fish in seapens unsuitable for marine anemia diagnosis based on linear regression analysis.

A) Full Model (p<0.001, C(p)=4, R^2 = 0.681)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>DIVE</td>
<td>+ 7.32</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>TEMP</td>
<td>- 2.35</td>
<td>0.46</td>
</tr>
<tr>
<td>MORTRATE</td>
<td>- 0.82</td>
<td>0.33</td>
</tr>
</tbody>
</table>

B) Final Model (p<0.001, C(p)=2, R^2=0.67)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>DIVE</td>
<td>+ 7.36</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

DIVE = frequency of mortality retrieval
TEMP = water temperature at 5 m.
MORTRATE = mortality rate for the week of the visit
Table 6.3. Correlation matrix of factors associated with the proportion of dead fish unsuitable for marine anemia diagnosis in seapens based on Pearson’s correlation coefficients

<table>
<thead>
<tr>
<th></th>
<th>POOR</th>
<th>DIVE</th>
<th>MORTRATE</th>
</tr>
</thead>
<tbody>
<tr>
<td>DIVE</td>
<td>+ 0.82*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MORTRATE</td>
<td>- 0.22</td>
<td>- 0.37*</td>
<td></td>
</tr>
<tr>
<td>TEMP</td>
<td>- 0.47*</td>
<td>- 0.50*</td>
<td>0.31*</td>
</tr>
</tbody>
</table>

* Denotes statistical significance (p<0.05)

DIVE = frequency of mortality retrieval
TEMP = water temperature at 5 m
MORTRATE = mortality rate for the week of the visit
suitable for diagnosis as well as the variation in the frequency of dead fish recovery.

Discussion

The proportion of total deaths attributable to a specific cause is an essential measure of the impact of a fatal disease in a population (Martin et al., 1987, p. 57). The results of this study show that, by performing necropsies on seapen mortalities, the marine anemia-specific mortality rate of farmed chinook salmon can be directly measured. The comparisons of disease specific mortality rates in different populations is a fundamental epidemiologic method used to associated causal or preventative factors with a disease, and are the foundation for the development of rational disease control and prevention programs (Martin et al., 1987, pp. 129-132).

By using mortality surveys, a substantially larger sample of the seapen population could be examined than when only surface moribund fish were sampled. Not surprisingly, the diagnostic quality of the dead fish was significantly influenced by the length of time that fish lay dead at the pen bottom. Recommending that divers remove all dead fish from pens 12-24 hours prior to collecting samples for a disease investigation is an important method of maximizing the quality of mortalities for diagnosing marine anemia,
regardless of the water temperature or farm mortality rate. Had farm managers ensured that all study pens been "dived" within 24 hours, the proportions of fish suitable for marine anemia diagnosis would have been significantly greater than the numbers encountered under "routine" dive schedules. The unexpected negative relationship between the proportion of dead fish suitable for marine anemia diagnosis and the water temperature may be an artifact resulting from the management practices of British Columbia salmon farms. The significant negative correlation between the frequency of mortality recovery and the water temperature may, in part, explain the negative coefficient associated with water temperature in the full regression model. In British Columbia, high water temperatures are frequently associated with seasonal management requirements that decrease the time farm staff have available for diving pens on chinook farms.

The results of this study suggest that past estimates of the prevalence of marine anemia at a farm or industry level, based only on samples of surface moribund fish, were probably underestimates. As mortality surveys detected marine anemia in a pen more frequently than did surface samples taken from the same pen, past estimates of the prevalence of marine anemia were likely conservative. The true prevalence of marine anemia, at an industry level, likely exceeds the past estimates of 6-20% that have been
derived from morbidity surveys of B.C. seapens (Brackett et al., 1991; Brackett et al., 1990).

The superior ability of mortality surveys to detect marine anemia in a seapen may result from the examination of a larger number of fish. Increasing the size of a sample relative to the size of the reference population increases the probability of the sample detecting a disease (Cannon and Roe, 1982). Mortality surveys should, therefore, detect marine anemia at a lower prevalence than samples taken from the surface of the water. However, the formulas used to estimate the probability of a sample to detect a disease (Cannon and Roe, 1982) assume a random component to the sampling methods that are used. Neither the mortality surveys nor surface samples were random samples. Mortality surveys are obviously biased towards identifying diseases that are fatal, while surface samples are potentially biased towards identifying chronic disease processes (Chapter 4; Brackett et al., 1991). Neither technique is therefore capable of determining the true prevalence of marine anemia in the general seapen population. Such an estimate would require the random sampling of the entire pen (Thorburn, 1992). Despite these potential biases and limitations mortality surveys appear to be a valuable method for screening a farm for marine anemia and for quantifying its contribution to pen mortality rates.

Although this evaluation of mortality surveys was
restricted to marine anemia, the results suggest that mortality surveys would benefit other farmed salmon disease research and monitoring programs. A number of specific causes of mortality, such as gastric bloat, predation and non-smoltification, can be readily recognized by gross pathological signs alone (Hicks, 1989; Kent, 1992). In addition, many of the important diseases of salmon that are definitively diagnosed by microbiological or microscopic features can often be tentatively diagnosed using historical and gross pathological information alone (Hicks, 1989; Kent, 1992). Despite the inability of mortality surveys to provide precise etiological or histopathological diagnoses for all cases, the routine examination of mortalities would allow fish health personnel to more accurately monitor changing patterns of the general causes of mortality in their seapens. By regularly monitoring these changing patterns, salmon farmers might gain new insight into the factors that influence not only the extent and impact of marine anemia, but also the epidemiological characteristics of other fatal diseases of seapen salmon.
References


Appendix 6.1. Relationship of post-mortem gill colour and quality of tissues for gross and histopathological examination.

<table>
<thead>
<tr>
<th>TISSUE QUALITY</th>
<th>N(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GOOD</td>
</tr>
<tr>
<td>WHITE</td>
<td>117 (35%)</td>
</tr>
<tr>
<td>LIGHT PINK</td>
<td>63 (50%)</td>
</tr>
<tr>
<td>DARK PINK</td>
<td>132 (81%)</td>
</tr>
<tr>
<td>RED</td>
<td>5 (100%)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>313</td>
</tr>
</tbody>
</table>
CHAPTER 7

DESCRIPTIVE EPIDEMIOLOGY OF MARINE ANEMIA IN SEAPEN-REARED SALMON IN SOUTHERN BRITISH COLUMBIA

Introduction

In 1988, a previously unrecognized disease of farmed chinook salmon (*Onchorhynchus tshawytscha*), known as marine anemia or plasmacytoid leukemia, was described in British Columbia, Canada (Kent *et al.*, 1990). In the past, marine anemia research concentrated primarily on the microbiological and pathological features of the disease. Experimental studies provided important evidence used to characterize marine anemia as an infectious neoplasm. The salmon leukemia virus, a recently described retrovirus, has been implicated as the cause of the disease (Eaton and Kent, 1992; Kent, 1992). Early field investigations suggested that marine anemia was the cause of extremely high mortality rates on commercial farms and, therefore, represented a threat to the economic viability of the British Columbia salmon farming industry (Brackett *et al.*, 1990; Newbound and Kent, 1991a). However, these early field studies were often limited in their ability to reliably
reveal important aspects of the impact and natural occurrence of the disease (see chapters 1, 2, 5). In many instances, information regarding the sources, transmission, and effects of marine anemia in British Columbia have been inferred from laboratory studies (Kent and Dawe, 1990; Newbound and Kent, 1991b; Kent and Dawe, 1993).

There are, in general, three approaches that are used to study disease: laboratory experiments, case series and population studies (Susser, 1973). Although laboratory experiments are capable of providing precise measurements of pathophysiological changes that occur in affected individuals, they are limited in their ability to replicate the complex ecological relationships responsible for maintaining and propagating a disease under natural conditions (Susser, 1973; Hanson, 1988). In contrast, population studies are chiefly concerned with describing aspects of the natural history of a disease. As most outbreaks of disease are a result of an imbalance in the ecological relationship of disease determinants (Martin et al., 1987), understanding a disease’s natural history is an important step in formulating rational prevention and control programs.

Epidemiological studies do not rely on any unique method but instead employ all available methods and tools to determine the distribution and dynamics of a disease (Sartwell and Last, 1980). Descriptive epidemiology often
involves the use of field observations to identify and characterize a disease problem (Schwabe et al., 1977). There are a variety of ways to collect descriptive epidemiological data, ranging from simple counts of dead animals, to sophisticated disease monitoring and surveillance programs. The choice of which specific data collection methods to employ is influenced by the availability and reliability of data, as well as by the purpose of the investigation (Sartwell and Last, 1980).

One of the principal objectives of descriptive epidemiological studies is to provide observations and questions that can act as the basis for the creation of disease hypotheses (Schwabe et al., 1977). The orderly progression from hypothesis, through experiment, to prediction is often emphasized as the hallmark of science (Buck, 1975). However, productive hypotheses are not formulated in the absence of preceding critical observation and thought (Platt, 1964). The primary goal of this research was to describe patterns of marine anemia’s behaviour and occurrence in British Columbia in order to assess previously derived inferences and hypotheses about the disease.

Materials and Methods

Three methods were used to describe the distribution and characteristics of marine anemia in southern British
Columbia between 1991-1993: mail surveys, case finding, and mortality surveys. Because many commercial salmon farms were often inaccessible or lacked important disease data, all three methods were needed to generate a reliable data set. Information derived from these three sources was synthesized to identify and verify the location of affected farms, the distribution of the disease at a provincial and farm level, potential risk factors for the disease, and the impact of marine anemia on commercial farms.

The criteria for diagnosing marine anemia created in chapter five of the thesis was used as the case definition for this study. For retrospectively collected data, the diagnosis was based on previously recorded descriptions of gross and histopathological signs. When available, histological samples were reviewed to confirm the diagnosis. Cases were not included in this study if supporting pathological evidence was unavailable.

Mail survey

A mail survey was used to determine the industry's perception of the distribution and impact of marine anemia, and to identify farms on which to focus later case finding efforts (Appendix 7.1). Forty-five chinook seafarms were selected by the use of a random number table from a complete list of all licensed sites in British Columbia in 1991. The selection of 45 farms would allow us to
determine, with 95% confidence if marine anemia was present at or below a prevalence of 5% of farms, if no respondent reported being positive for the disease (Martin et al., 1987; p. 37). The list of licences was provided by the licensing agency for the industry, the British Columbia Ministry of Agriculture, Fisheries and Foods. The survey involved 41% of the active chinook production sites, representing 44% of the salmon farming companies in the province. The survey was pretested on three farm managers and two aquaculture veterinarians to insure that the intent of the questions were understood and that vital questions had not been neglected. Based on the pre-test, the questionnaire was redesigned and mailed to salmon farmers in November, 1991. This was followed by repeat mailings to non-respondents in January and March of 1992. Areas of inquiry included in the survey were: demographic characteristics of the farm, water quality, the basis for marine anemia diagnosis, the perceived pattern of occurrence, and the estimated impact of the disease.

Case finding

The characteristics of populations positive for marine anemia were established by assembling a series of positive fish from three sources: veterinary records, diagnostic laboratory records and samples obtained by the primary investigator during farm visits. From 1987-1992, veterinary
services were supplied to the British Columbia salmon farming industry by veterinarians employed by four companies providing feed or pharmaceuticals to the industry. Two of these companies, selected partially on the basis of convenience and cooperation, provided clinical records for review. The records of the other two companies were not utilized because of an insufficient level of detail in the medical records or because of the limited number of farms and geographic regions visited by their veterinarians. The records represented all of the clinical data collected by these companies between 1987-1992, and encompassed all of the major fish farming regions in the province.

The second source of retrospective data came from a review of diagnostic submissions to two fish health laboratories. Both the British Columbia Animal Health Centre in Abbotsford and the federal Fish Pathology Laboratory in Nanaimo provided access to their diagnostic records from 1987-1992. These laboratories were the primary diagnostic facilities used by the British Columbia salmon farming industry for the period of the study. The original histological tissue sections were available for review in approximately 90% of the cases derived from the federal laboratory, but in less than 10% of the cases obtained from the provincial laboratory.

Prospective "case finding" was the third technique
used to characterize the features of marine anemia positive farms. Between 1991 and 1993, 53 farm visits, involving 20 different farms, were conducted in southern British Columbia. Sites were selected on the basis of owner cooperation and accessibility. Efforts were made to visit sites previously identified in the analysis of clinical and laboratory records and by the results of the mail survey. At each visit, surface catchable moribund fish were collected from chinook pens. Fish were euthanized by cervical severance and subjected to gross pathological examination. Samples of multiple visceral organs were collected from each fish and stored in Davidson’s solution (Humanson, 1979). These tissues were later routinely prepared and stained with hematoxylin and eosin for histopathological examination (Humanson, 1979). During farm visits, water temperature, dissolved oxygen, water flow, and water clarity were measured at 1 m, 5 m, and 10 m depths inside and outside of the sampled netpens. The age and length of time at sea were recorded for all fish that were examined. In addition, the mortality rate for the week of a visit and concurrent disease problems were described and quantified on each farm visit. Mortality rates were obtained from fish farm records. Farm managers calculated the rate by dividing the number of dead fish recovered from the study pens for a week by the estimated number of fish in the pens. Estimations of the total pen population were
based on periodic complete counts of the fish and were
adjusted over time for the total number of dead fish
recovered from the pens.

In addition to farmed fish, wild chinook and sockeye
(Oncorhynchus nerka) salmon were collected by commercial
trolling or gill netting in the Strait of Georgia, and from
four spawning streams on Vancouver Island and the Sunshine
Coast (Figure 7.1). Sites for wild fish collection at sea
were selected on the basis of accessibility, and proximity
to seafarms that were positive for marine anemia. Specific
spawning streams were selected for sampling because the
strain of chinook present in these streams had historically
provided the parents of affected farmed fish. As with the
farmed fish, wild salmon were subjected to gross and
histopathological examination.

Mortality survey

Six commercial chinook farms were selected, on the
basis of accessibility and owner cooperation, for visits at
two to four week intervals, for six months. Farm visits
were conducted between June and December in 1992 and 1993.
At each visit, all surface catchable fish and mortalities
were retrieved from four adjacent seapens. Fish were
subjected to gross and histopathological examination using
the same protocols as in the case finding exercise. The
marine anemia status of dead fish that were too severely
Figure 7.1  General location of fish capture in southern British Columbia for descriptive epidemiologic study of marine anemia.

- sites of farmed fish collection
- sites of wild fish collection
autolysed for histological examination was determined on the basis of gross pathological lesions (see chapter 5 for the criteria for gross pathological diagnosis of marine anemia). All necropsied fish were placed into one of five diagnostic categories; (1) marine anemia, (2) productive inflammation, (3) non-productive inflammation, (4) non-infectious processes, and (5) open. Details of these diagnostic categories can be found in chapter two of the thesis. Water temperature at 5m depth was also recorded on each visit. Farm managers provided the mortality records for the sampled groups for the six month period of the study.

Pearson’s correlation coefficient (Colton, 1974, pp. 207-214), and the chi-squared test for association (Colton, 1974, pp. 175-179) were used to help describe the relationship of marine anemia with management, environment, and disease variables in the mortality survey and the case finding exercise. Inferences regarding the significance of the calculated correlation coefficients (r) were based on table values in Colton (1974, p. 349).

Results

The results of our mail survey revealed that, at the time of this study, chinook salmon farmers in British Columbia considered marine anemia to be a threat to their industry. The majority of the respondents (n = 27/38)
believed that marine anemia had a significant negative impact on affected farms, primarily by increasing mortality rates and decreasing growth rates. The survey suggested that, once detected on a farm, marine anemia became a chronic problem that was repeatedly diagnosed throughout the remainder of the production cycle, as well as in subsequent years.

Marine anemia had been diagnosed on 26% of chinook farms that responded to the mail survey (n=10). However, only six of these positive farms used a case definition that was consistent with the diagnostic criteria selected for this study. Each of these six sites relied upon the histopathological evaluation of multiple tissues by an experienced fish pathologist as the criteria for determining the marine anemia status of their farms. Other positive farms used case definitions that were inconsistent with the criteria used in this study for diagnosing marine anemia. Three of these positive sites diagnosed the disease whenever microsporidial parasites were detected in liver tissue imprints. The remaining positive farm considered the finding of large, immature, mononuclear cells in Giemsa stained liver imprints sufficient evidence for diagnosing marine anemia. After classifying farms using inadequate case definitions as negative for the disease, the survey suggested a farm level prevalence of marine anemia of 19% (6/32) of operating chinook sites in British Columbia.
Marine anemia was eventually diagnosed on the all but one of commercial chinook farms visited during this study. Follow-up visits conducted on ten of the farms that participated in the mail survey found cases at each farm, including five sites that had been classified as negative for the disease in the survey. Similarly, of the 23 farms visited during the mortality surveys and case finding program, only one failed to reveal positive cases. This farm remained negative for the disease for twelve visits over six months. Prior to this project, marine anemia had not been diagnosed on 15 of these 23 farms. The proportion of total mortality attributed to marine anemia during mortality surveys of positive farms ranged from 2.5% to 11% (mean = 6%)

Although diagnosed on virtually all of the farms involved in the study, marine anemia could not be found in all chinook pens on affected farms. In addition, the disease was not found on every visit to an affected farm. On average, two visits were required per site before the disease was detected by mortality or morbidity surveys. The frequency of detecting the disease on a farm varied from only once in ten visits, to eleven of twelve visits. On approximately one half of the farms, marine anemia could only be sporadically detected. For example, cases of the disease would be found on one visit, then were not diagnosed on the two subsequent visits, before being
diagnosed again on a forth farm call.

Due to the potential risks of producer misclassification of the marine anemia status of their farms because of differing case definitions and potential under-reporting, information regarding risk factors for marine anemia obtained from the mail surveys was judged to be unreliable. Therefore, potential risk factors were sought from the retrospective analysis of diagnostic records and from on-farm investigations.

As most of the retrospectively obtained cases of marine anemia were reported in farmed-reared, monosexed chinook (fish chemically or physically altered while at the age stage to be phenotypically female) that had been at sea for over one year, the historical data suggested that species, age, and sex factors were potentially associated with the disease. However, marine anemia was not diagnosed exclusively in this particular demographic group (Table 7.1). Isolated cases of marine anemia were reported in clinical and laboratory records in farmed Atlantic salmon (*Salmo salar*), farmed coho salmon (*Oncorhynchus kisutch*), farmed sockeye, and wild chinook. The diagnosis was confirmed in each of these groups by reviewing the original tissue sections. Although the laboratory records documented positive cases in wild chinook, it was not possible to determine if these fish were truly wild or were fish farm escapees. A single report in the clinical records
<table>
<thead>
<tr>
<th>Mortality Survey</th>
<th>Case Finding (Wild salmon)</th>
<th>Case Finding (Farmed salmon)</th>
</tr>
</thead>
<tbody>
<tr>
<td>31</td>
<td>788</td>
<td>482</td>
</tr>
<tr>
<td>0</td>
<td>294</td>
<td>294</td>
</tr>
</tbody>
</table>

**Prospective**

<table>
<thead>
<tr>
<th>Laboratory Records</th>
<th>Clinical Records</th>
</tr>
</thead>
<tbody>
<tr>
<td>163</td>
<td>1212</td>
</tr>
<tr>
<td>19</td>
<td>733</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Source of Samples</th>
<th>Number of salmon</th>
<th>Number of marine samples</th>
<th>Number of marine</th>
<th>Number of wild Pacific salmon</th>
</tr>
</thead>
<tbody>
<tr>
<td>252</td>
<td>2809</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 7.1: Sources of marine anemla cases in farmed and wild Pacific salmon
described a rainbow trout (*Oncorhynchus mykiss*) with histopathologic features consistent with the disease. However, tissue samples were not available to confirm the diagnosis.

During farm visits, cases of marine anemia were found in male, female, and monosexed fish. The age of affected fish ranged from S3 smolts that had been at sea for approximately four months, to broodstock that had been at sea for four years. Monosexed chinook that had been at sea for one year were likely over-represented in the historical records because, at the time of this study, these were the predominant type of fish farmed in British Columbia, and, due to their high input value, chinook producers were particularly concerned about the disease in this group of fish.

No single source could be implicated as the origin of marine anemia. Hatcherries providing progeny that were later diagnosed with the disease were located throughout Vancouver Island and the southern mainland of British Columbia. Both well water and surface water hatcherries provided stock to affected farms. Different strains and lineages of broodstock were used by different positive farms. Broodstock originated from spawning grounds throughout Vancouver Island, the southern and northern coasts of British Columbia, and the Yukon. Some affected fish were first generation offspring of wild salmon, while
others were commercial "domesticated" stock.

The occurrence of marine anemia on commercial chinook farms appeared to follow a distinct seasonal pattern. Although cases of the disease could be found throughout the year, the number of cases of marine anemia detected during the farm investigations and in diagnostic records peaked in September in each year of the study (Figures 7.2 and 7.3). However, other significant events also occurred in September. First, water temperatures began to decline from yearly maximums at this time of the year. The correlation between the proportion of mortality attributed to marine anemia and the water temperature on the day of the mortality survey was positive, but statistically insignificant ($r=0.31$, $p>0.05$). Second, there was a shift in the pattern of diseases seen in the seapens. The frequency of diagnosing productive inflammatory processes was lowest during September, while the number of diagnoses of non-productive inflammatory diseases peaked near this time of year.

The pattern of disease varied not only seasonally, but also between farm visits. There was a significant difference in the pattern of mortality seen on farm visits when marine anemia was detected compared to visits when the disease was not diagnosed (chi-squared = 40.62, $p<0.0001$). On average, there was a higher proportion of deaths attributable to non-productive inflammation, and a lower
Figure 7.2. Seasonal distribution of the proportion of submissions to veterinarians and diagnostic laboratories diagnosed as marine anemia. (1987-1992).
Figure 7.3. Seasonal distribution of the proportion of fish sampled diagnosed as marine anemia in field studies.
proportion attributed to failure to adapt to sea water (non-smolts) and open diagnoses on days when marine anemia was diagnosed than on visits on which it was not (Table 7.2). On farms that were monitored for six months, the increase in the number of deaths attributed to non-productive inflammatory processes coincided with a rise in the proportion of deaths due to marine anemia in each year of the study.

The detection of marine anemia on a farm was preceded by an increase in the number of fish demonstrating hyperplasia of the cells in the caudal renal interstitium as their only significant lesion. These hyperplastic lesions had a lower proportion of blast cells and a scarcity of mitotic figures as compared to fish with marine anemia. Also, these cases lacked blast cell infiltration into tissues other than the kidney. This lesion was more frequently identified on visits when marine anemia was found than on farm visits when no marine anemia was detected.

The pattern of mortality seen over six months at the single negative farm was notably different than that of a neighbouring positive farm. Located less than one km from each other, both farms introduced one year old chinook smolts to sea in the spring of 1993. By late August of 1993, marine anemia had been diagnosed at one of these farms. This farm continued to provide cases of the disease
<table>
<thead>
<tr>
<th></th>
<th>Positive Anemia</th>
<th>Negative Anemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marline Open</td>
<td>13%</td>
<td>33%</td>
</tr>
<tr>
<td>Process disorders Non-Productive Non-Infectious</td>
<td>26%</td>
<td>7%</td>
</tr>
<tr>
<td>Non-Smokers</td>
<td>14%</td>
<td>8%</td>
</tr>
</tbody>
</table>

Table 7.2. Mean prevalence of diagnostic categories observed during mortality surveys on chinook (Oncorhynchus tshawytscha) seafarms.
until the end of October. Over the six month surveillance period, 80% of deaths at the negative farm were attributed to predation and non-smolts, whereas at the positive farm inflammatory processes were the most frequently diagnosed disease process (45%). However, the six month cumulative mortality rates at these two farms were not significantly different.

Despite differences in the proportional mortality rates between farm visits on which marine anemia was detected and those on which it was not, the weekly cumulative mortality rates recorded on visits during which marine anemia was found were not significantly different than for visits on which the disease was not diagnosed (chi-squared = 0.39, p>0.5). Although the prevalence of marine anemia seen in mortality surveys was positively correlated with pen mortality rates (r=0.46, p<0.05), high mortality rates were recorded on positive and negative farms. None of the farms sampled in this study suffered "devastating" rates of mortality at the time of marine anemia diagnosis. In both the mortality survey and the prospective case finding exercise, the diagnosis of marine anemia was associated with decelerating mortality rates slightly more often (52%, n=12) than it was with accelerating rates (44%, n=10).
Discussion

Marine anemia was not described in farmed salmon in British Columbia until the fall of 1988 (Kent et al., 1990). Within four years of first being described, the disease had been diagnosed in four of the five major salmon farming regions in the province and had been declared a major threat to the local aquaculture industry (Anon., 1992; Chapter 2). The sudden occurrence of a disease that was previously unrecognized is not uncommon (Ampel, 1991). Several factors have been proposed to explain this phenomenon: a disease may have been present all along, but previously unrecognized or mis-classified; a new, virulent organism may be introduced into a susceptible population; a "new" disease may be the result of changes in the structure or ecology of an "old" pathogen; or environmental or behavioural factors may alter the severity or likelihood of a disease occurring (Ampel, 1991; Martin et al., 1987). Understanding the relative importance of the factors responsible for the "emergence" of a disease is essential, not only for determining the causes and impact of an apparently new disease, but also for the rational development of control and prevention programs.

The rapid growth of the chinook farming industry in British Columbia in the 1980's may have contributed to the "discovery" of marine anemia. By providing large numbers of readily accessible moribund salmon, large scale salmon
farming undoubtedly increased the probability of detecting previously unrecognized diseases, including marine anemia. Although evidence presented in this study suggests that marine anemia is present in wild stocks, problems in sampling wild salmon would have undoubtably complicated the detection and description of marine anemia in these fish. Even in farmed salmon, problems have been encountered in consistently diagnosing the disease (Chapter 4). As late as 1990, inconsistent mortality patterns and concurrent infections inhibited the ability of investigators to identify marine anemia as a distinct syndrome (Brackett et al, 1990; Newbound et al, 1993). The lack of a consistent case definition used by fish farmers also increases the probability that earlier cases of marine anemia would not have been categorized as part of a specific syndrome. Alternatively, problems in diagnosing the disease at the individual and group level creates the possibility that, by repeatedly visiting a site and testing for marine anemia, false positive diagnoses may have been encountered. Until a more reliable method of determining the marine anemia status of fish and farms is developed, more precise measurements of risk factors associated with the disease will be difficult to achieve.

The environmental conditions created by intensive aquaculture may have also facilitated the emergence of marine anemia. Rearing systems used in seapen aquaculture
represent a substantial change in the ecology of chinook salmon (Chapter 2). Increasing farm sizes, accelerated feeding schedules, stress of shipping and confinement, and sub-optimal environmental conditions may all contribute to the susceptibility of farmed salmon to infectious disease, as well as maximizing opportunities for the propagation and maintenance of diseases on farms (Peterson et al., 1991, Griffiths and Warren, 1983). As large scale production of chinook salmon did not truly begin until the mid-1980's (Anon, 1992), opportunities for the occurrence of marine anemia may not have been present before that time. If marine anemia is a "disease of confinement", then its discovery may simply be a reflection of the recent rapid growth of the British Columbia salmon farming industry.

The diagnosis of marine anemia soon after fish have been put to sea suggests that the disease is either rapidly acquired after introduction to saltwater, or that fish arrive at sea farms with the disease. The horizontal spread of a potential marine anemia pathogen at sea would be facilitated by the intimate contact and frequent mixing of stock that occurs on salmon sea farms in British Columbia. The ubiquitous nature of marine anemia seen in this and other studies (Chapter 2) would, however, suggest that if acquired at sea, factors responsible for causing marine anemia must be widespread throughout the southern coast of British Columbia. Use of mixed sources of replacement stock
in the industry presents the prospect that a pathogen may initially be restricted to selected stocks but, upon sea entry, is rapidly disseminated through susceptible populations. In the latter case, contributing causes or predisposing factors for marine anemia would have to be encountered while fish are still in fresh water. The identification of syndromes histologically indistinguishable from marine anemia in freshwater chinook salmon in California supports the hypothesis that marine anemia is not restricted to the salt water phase of salmon rearing (Harshbarger, 1984; Hedrick et al, 1990; Morrison et al, 1990; Kent and Dawe, 1993).

The source of an infectious agent for marine anemia in fresh water is unclear. Management practices employed by many of the hatcheries that provided positive stock minimized the opportunities for the perpetuation of a pathogen by horizontal transmission. The use of well water, which is generally considered free of wild fish and their obligate pathogens, the surface disinfection of eggs, and the separation of stocks and year classes were techniques that many of the hatcheries identified in this study used to reduce the horizontal spread of pathogens. As viruses responsible for infectious leukemias in other species can be vertically transmitted (Payne, 1987; Burny et al, 1980), vertical transmission is an alternative route for the spread of a marine anemia pathogen in hatcheries. The
tremendous range of sources of broodstock producing positive progeny suggest that, if vertically transmitted, a marine anemia pathogen must be widespread in British Columbia salmon populations and is not restricted to chinook salmon. The broad demographic distribution of marine anemia, in combination with its endemic nature, suggest a long-term relationship between the host and agent, and not the recent introduction of a new pathogen (Topley, 1942; Ewald, 1994).

Historically, estimates of the "experience" a population has had with a disease has been inferred from the impact the disease had on the population. With infectious disease, it has been hypothesized that a pathogen evolves towards benign coexistence with their hosts (Ewald, 1994). The excessive mortalities seen in previous field observations of marine anemia coupled with the results of experimental transmission studies caused investigators to infer that the disease was far from benign (Kent et al, 1990; Kent and Dawe, 1993). The excessive mortality rates associated with marine anemia, therefore, suggested marine anemia may be a new disease. However, the observations collected in this study did not support this inference. Although farms with increased mortality rates did have marine anemia, the disease rarely contributed to more than 10% of the total number of dead fish in an affected pen. In addition, the diagnosis of marine anemia
was associated with decreasing rates of mortality as often as it was associated with increasing rates. The failure of past field studies to examine mortalities when investigating natural outbreaks of the disease, and the extrapolation of the results of laboratory transmission studies may, in part, explain the differing interpretations of the impact of marine anemia on salmon farms. As recent theoretical and empirical studies have challenged the concept of "obligate evolution to benignness" (Ewald, 1994) resolving these conflicting views of the impact of marine anemia will not likely help us to decide if marine anemia is in fact a new disease.

The viral induction hypothesis of carcinogenesis has steadily gained support because of the frequent isolation of viruses from human and animal tumours (Gross, 1983). However, as many of these "tumour viruses" were also found to be widespread in healthy animals and humans, the sufficiency of retroviruses to act as the sole cause of neoplasia has been questioned (Duesberg, 1987). The association of marine anemia with a variety of immune stimulating factors, such as higher water temperatures and concurrent infectious disease, suggests that immunostimulation may play an important role in the pathogenesis of the disease. A major difference in the immune response of mammals and fish is the effect of environmental temperature on the latter. At low
temperatures, fish are immunosuppressed, whereas higher water temperatures facilitate cell mediated and humoral immune responses (Corbel, 1975). Peaks in the number of cases of marine anemia diagnosed in this study occurred when water temperatures facilitated the progression of infectious diseases and allowed a maximum immune response (Groberg et al, 1978). In addition, peaks in the prevalence of marine anemia in mortality surveys were associated with specific and non-specific signs of inflammation. The only farm at which marine anemia was not diagnosed was also the only farm at which infectious and inflammatory disease contributed little to the overall mortality rate.

The role of an active immune response and concurrent infection in the pathogenesis of leukemia has been suggested elsewhere. For example, an immune response to the presence of a retrovirus, as opposed to actions of the virus itself, has been proposed as a cause of chronic lymphocytic leukemia in man (Foon et al, 1990). Alternatively, exogenous infections with other agents may act as a necessary stimulating mechanism for initiating the transcription and proliferation of endogenous retroviruses responsible for neoplastic disorders (Murray et al, 1990). Ongoing challenges to the immune system in the form of concurrent disease, may therefore be hypothesized to be an important contributing cause of marine anemia, especially when environmental conditions facilitate a maximal immune
response.

Although the association of experimental models of marine anemia with the salmon leukemia virus supports the hypothesis that marine anemia is a specific, virus induced disease, it does not preclude the alternative hypothesis that marine anemia is not a specific disease, but is instead a non-specific immune response. It should be remembered that a disease is the effect of a pathological process on a patient and that the pathological process must not be confused with the cause of the disease (Scadding, 1988). A consistency of pathological lesions does not equal a consistent cause for the lesion. For example, the chronic inflammatory response associated with delayed-type hypersensitivity reactions is consistently characterized by tissue infiltration by mononuclear cells and generalized hyperplasia of lymphatic organs, yet the inciting cause of these reactions can be a variety of infectious agents (Suter, 1990). In the case of marine anemia, other infectious organisms, such as Enterocytozoan salmonis and Renibacterium salmoninarum have been associated with the disease (Kent et al., 1990; Brackett et al., 1990; Newbound and Kent, 1991). Because of the reliance on post-mortem lesions to diagnose marine anemia (Kent et al., 1990), it has not yet been possible to directly describe the sequential pathology of this syndrome in individual fish and thus establish the temporal relationship of potential
causal factors with the occurrence of the disease. Non-lethal, reliable tests for agents such as a salmon leukemia virus will be required to elucidate the role of infectious agents in the pathogenesis of marine anemia.

Descriptive epidemiological studies are better suited to generating than testing hypotheses (Schwabe et al., 1977). Natural experiments, such as case control or cohort studies, in combination with ongoing laboratory investigations will be required to test the hypotheses that have been developed in this study. If immunological cofactors are found to be important in the genesis of marine anemia, new avenues for disease control and prevention may be found. These results suggest that the importance of diagnosing marine anemia on a salmon farm is not that it predicts impending epidemics of mortality, but instead the detection of marine anemia may be an important indicator of the pattern of disease on affected farms.
References


Appendix 7.1. Marine anemia mail survey sent to randomly selected licensed chinook salmon seafarms in British Columbia

MARINE ANEMIA SURVEY

(identification code)

SECTION #1:
OCCURRENCE OF MARINE ANEMIA ON YOUR FARM

A) Has marine anemia been diagnosed at your farm?
   Yes ___  No ___
   - if you answered no, go to section #2 of the survey

B) How many pens were affected on your site?
   - number of pens affected ________
   - total number of pens on the site ________

C) How did you first notice that marine anemia was at your farm?

D) Was the diagnosis of marine anemia confirmed by lab tests?  Yes ___  No ___
   - if yes, list the tests that were used

_________________________________________________________________
E) In what years has marine anemia been diagnosed at your farm?

F) Do/did you find more cases of marine anemia at a certain time of the year? Yes ___ No ___
   - if yes, when: ____________________________
   ____________________________
   ____________________________

G) Have you noticed any of the following patterns for marine anemia on your farm? (Circle the best answer)
   1) see cases of the disease "now and then"
   2) cases began to appear, the number peaked, and then no more cases were found
   3) cases began to appear and then could routinely been found
   4) no pattern noticed
   5) other(describe) ____________________________
       ____________________________
       ____________________________

H) On your farm, is/was the disease found in (Circle the best answer)
   1) fish in only one pen
   2) all pens on the site
   3) only certain groups of fish (describe)
       ____________________________
       ____________________________
       ____________________________

I) How does/did marine anemia affect your mortality rates? (Circle the best answer)
   1) no effect
   2) mortalities decreased
   3) mortalities increased
J) How does/did marine anemia affect your production?  
   (Circle the best)
   1) no change
   2) increased
   3) decreased

K) Has marine anemia affected the occurrence of other diseases on your farm? (Circle the best answer)
   1) no change
   2) less of other diseases
   3) more of other diseases
   - please describe the change ____________________________

L) Were the fish with marine anemia; monosexed___ or regulars ___?

SECTION 2
FARM INFORMATION

A) Approximately how close is your site to other salmon farms? (Circle the best answer)
   1) 1 km
   2) 5 kms
   3) 10 kms
   4) 20 kms or more

B) Approximately how many fish do you market from this site in a year?

___________________________________________
C) How would you describe your site? (Circle the best answer)
   1) deep water, good water flow
   2) deep water, poor water flow
   3) shallow water, good water flow
   4) shallow water, poor water flow

D) Do you mix year classes at this site?
   Yes _____  No _____

E) Do you get your smolts from this site from a single source?
   Yes _____  No _____

COMMENTS

Thank-you for participating in this survey of marine anemia.
Appendix 7.2. Morbidity and mortality information from adjacent "matched" seafarms. Farm A = marine anemia positive. Farm B = marine anemia negative.

<table>
<thead>
<tr>
<th>MONTH</th>
<th>FARM</th>
<th>RATE</th>
<th>MA</th>
<th>PROD</th>
<th>NP</th>
<th>NI</th>
<th>OPEN</th>
</tr>
</thead>
<tbody>
<tr>
<td>June</td>
<td>A</td>
<td>1.99</td>
<td>0</td>
<td>33</td>
<td>2</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.14</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>July</td>
<td>A</td>
<td>0.33</td>
<td>0</td>
<td>18</td>
<td>0</td>
<td>80</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.18</td>
<td>0</td>
<td>12</td>
<td>2</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.18</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Aug.</td>
<td>A</td>
<td>0.15</td>
<td>1</td>
<td>1</td>
<td>18</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.07</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Sept.</td>
<td>A</td>
<td>0.16</td>
<td>2</td>
<td>1</td>
<td>6</td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>0.16</td>
<td>0</td>
<td>21</td>
<td>0</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.08</td>
<td>0</td>
<td>18</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.21</td>
<td>0</td>
<td>4</td>
<td>6</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Oct.</td>
<td>A</td>
<td>0.14</td>
<td>3</td>
<td>17</td>
<td>5</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.05</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Nov.</td>
<td>A</td>
<td>0.14</td>
<td>0</td>
<td>3</td>
<td>9</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>0.13</td>
<td>0</td>
<td>18</td>
<td>6</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.35</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.73</td>
<td>0</td>
<td>4</td>
<td>1</td>
<td>87</td>
<td>1</td>
</tr>
</tbody>
</table>

Rate = weekly mortality rate for yearclass of fish studied  
MA = number of marine anemia diagnoses made  
Prod = number of fish classified as productive infections  
NP = number of fish classified as non-productive infections  
NI = number of fish classified as non-infectious diseases  
Open = number of fish with open diagnoses
CHAPTER EIGHT

CONCLUSIONS

The new perspective of marine anemia that was generated by studying the disease on salmon farms conflicted with many of the previously held views about the occurrence, behaviour, and impact of the disease in British Columbia. Unlike past reports based primarily on laboratory studies and limited surveillance programs, I could not conclude that marine anemia was an infectious neoplasm that caused epidemics of mortality on chinook salmon seafarms (Kent et al., 1990; Newbound and Kent, 1991a; Newbound et al., 1993). Instead, when studied in a population and ecological context, marine anemia appeared to be an endemic syndrome. No evidence could be found to suggest that this syndrome was a spreading "cause" of epidemics of mortality. Evidence gathered in this study also challenged the classification of marine anemia as a new, distinct disease caused by a single, specific pathogen.

In the midst of conflicting interpretations of the natural history and significance of marine anemia, the disease continues to be diagnosed. In British Columbia, fish health professionals continue to be solicited for
advice on how to control or prevent the occurrence of the disease. It is the intent of this chapter to review evidence gathered in this and other research in order to evaluate options for reducing the impact of marine anemia on salmon farms.

Epidemiology has been described as an exercise in observing and measuring a disease under the conditions in which it normally exists (Rothman, 1986 p. 2). The major purpose of epidemiological studies is to provide information on which rational decisions for the prevention and control of a disease in a population can be based (Martin et al, 1987 p. 7). The quality of the decisions that arise from a study are dependent on the quality of the measurements made. A brief review of the factors that may have influenced marine anemia research is, therefore, required before preceding to evaluate intervention options for the disease.

The accuracy of an epidemiological study can be affected by random and systematic errors (Rothman, 1986, p. 77). Whereas random errors are a reflection of the unpredictability of biological systems, systematic errors can arise from the inappropriate selection of study subjects, and from uncontrolled or unanticipated factors that distort our observations and measurements (Rothman, 1986, pp. 77-97). Although unexplained variation created by random error has likely influenced marine anemia research,
I believe that systematic errors were primarily responsible for generating differing interpretations of the nature of the disease.

Prior to this project, most studies of marine anemia focussed primarily on the "tail end" of the spectrum of the disease. On an industry level, surveys for marine anemia were frequently initiated only after the disease had been detected in a region (Chapter 2); in netpens, sampling for marine anemia relied principally on obtaining terminally or chronically ill fish for examination (Chapter 3); and at the individual level, the diagnostic features of the disease were based solely on the signs and lesions of dead and dying fish (Chapter 4 & 5). A valid generalization of the results of a disease investigation requires the study population to be representative of the population of concern (Sox et al, 1988, p. 117). Differences in the range of clinical presentation and severity of marine anemia between groups of fish that have been studied and the general population of commercially farmed salmon are the primary reasons of the differing interpretations of the nature of marine anemia on salmon farms. Although the study subjects chosen for this research were not representative of the British Columbia salmon farming industry as a whole in a purely statistical sense, they were selected so that a larger portion of the spectrum of the marine anemia could be observed. By investigating marine anemia in its natural
setting, this research has provided a clinically relevant framework into which the results of field and laboratory studies can be incorporated. Such a synthesis of information will improve our understanding of the factors responsible for the propagation and maintenance of marine anemia on salmon farms.

Medicine is said to be "the art of making decisions without adequate information" (Sox et al., 1988, p. ix). An incomplete understanding of the etiology of marine anemia does not prevent one from developing effective plans for its prevention or control. The history of medicine has shown that many diseases can be effectively managed before the entire web of causation has been revealed. The control of cholera in 1854 by restricting access to water contaminated with faeces, the use of citrus fruit to prevent scurvy in sailors, and the prevention of congenital joint laxity and dwarfism in beef calves by managing the feeding of pregnant cows are just three examples of preventive programs that were employed successfully to control diseases before their causes were fully understood (Cameron and Jones, 1983; Mauser and Bahn, 1974; and Ribble et al., 1989). Although controversy regarding marine anemia continues to exist, the study of the disease on farms has provided a broader base of information from which hypotheses regarding the control and prevention of marine anemia can be generated.
Schwabe et al (1977) described seven "directed actions" for controlling disease in animal populations: selective slaughter, depopulation, quarantine, mass immunization, mass treatment, environmental manipulation, and public education. The choice of which of these actions, or combination of actions, are applicable for the control of a specific disease requires careful consideration of biological and economic aspects of the disease (Martin et al, 1987, p. 255). For the remainder of this chapter, I will explore these alternative disease management options, with respect to marine anemia, based on the understanding of the disease developed in this research.

The deliberate killing of animals labelled as "affected", whether done selectively or by the complete destruction of entire groups, has been used successfully to eradicate or prevent the spread of a number of animal diseases (Schwabe et al, 1977). The objective of depopulation or selective slaughter programs is to interfere with the natural history of a disease so as to make its propagation unlikely (Martin et al, 1987, p. 250). Diseases that are susceptible to control by selective slaughter or depopulation share two main features: the cost of the program is offset by the potential or real impact of the disease, and the disease can be reliably diagnosed and detected (Martin et al, 1987, p.250-251).

The selective slaughter of individual fish with marine
anemia is an oxymoron as, to diagnose the disease, the individual must be dead. Slaughter programs to control marine anemia would, therefore, be aimed at the group level. However, the wide spatial and host distribution of marine anemia, coupled with its endemic nature, would result in the destruction of large numbers of fish in British Columbia if industry-wide depopulation programs were employed to control the disease. As marine anemia generally contributed to less than 10% of the total number of dead fish on affected farms (Chapter 7), the depopulation of affected pens would result in the destruction of a substantially larger number of fish than would the disease. The lack of regulations that provide for the financial compensation of farmers whose fish have been destroyed in disease control programs suggest that attempts to depopulate salmon farms to control marine anemia would result in strong opposition from the aquaculture industry, and a corresponding under-reporting of the disease.

To determine who should be slaughtered, individuals or groups must be correctly classified as having or not having the disease. As a disease is an evolving process, the point at which an individual or population should be labelled as "diseased" may be arbitrary or difficult to define (Mausner and Bahn, 1974). Problems in correctly classifying the marine anemia status of pens or farms would present a significant obstacle to the successful implementation of
slaughter programs (Chapter 3 & 4). Because of their inconsistency and uncertainty, previously applied diagnostic decision rules for marine anemia would be inadequate for determining the fate of many salmon farms (Chapters 4, 5, 7). By developing a method of detecting marine anemia in moribund and dead fish, this study developed a more consistent diagnostic rule that reflected a larger portion of the pen population. However, we are still unable to detect the disease in any stage other than its terminal presentation. As the characteristics of the screening test used play a major role in the effectiveness of slaughter programs, one would have to advise caution in applying depopulation programs to control marine anemia using the current methods that are available to diagnose the disease. Therefore, for economic and diagnostic reasons, selective slaughter or depopulation programs are unlikely to be effective for the control of marine anemia on salmon farms.

Restricting the movement of fish on farms where marine anemia is diagnosed has been suggested as a means to restrict the spread of the disease (Kent, 1993). In such a quarantine program, fish from affected farms would only be moved from their "home" sites at harvest. By preventing the contact of affected fish with apparently healthy fish, quarantine programs would attempt to minimize the spread of infectious agents associated with the disease. Aspects of
the natural history of marine anemia uncovered in this study weaken the argument for using quarantine programs for the control of the "spread" of marine anemia. The value of quarantine programs would be restricted by problems in determining the "true" marine anemia status of a farm, by our inability to detect the disease early, and by the uncertainty of the role of infectious agents in the etiology of the disease.

As a general rule, the number of animals affected by less severe forms of a disease outnumber those that are severely ill or dying because of the disease (Martin et al., 1987, p.5). As the salmon farming industry generally relies upon the examination of dead or dying fish to determine the marine anemia status of a pen or farm (Chapter 3), populations demonstrating early or sub-clinical forms of marine anemia would likely go undetected. Although it is conceivable that by restricting the movement of populations exhibiting "fulminant" marine anemia the overall "infection rate" of the industry may be reduced, our inability to confidently classify the disease and infection status of a farm would complicate efforts to coordinate and regulate fish movement for controlling the spread of a marine anemia pathogen.

Although quarantine may provide for physical separation of "positive" and "negative" farms, the quarantine of pens or year-classes on a farm would be
virtually impossible. The intimate interaction of fish with their aqueous environment, the shifting tidal flows of water, the crowded conditions of seapens, and the frequent movement and mixing of groups of fish on a farm, dramatically reduce the probability of preventing the horizontal transmission of pathogens on salmon farms. As most of the chinook farms in British Columbia have overlapping year-classes, the use of quarantine to prevent the spread of a pathogen would effectively force farmers to leave sites fallow for a year in order to break the cycle of transmission. Once again, the potential financial hardships such a program would impose on the industry would result in poor compliance with quarantine programs on the part of many farmers.

Once a virus was proposed as the cause of marine anemia (Eaton and Kent, 1992), mass immunization programs became a theoretical means to control the disease. Unfortunately, it has yet to be established that a virus causes marine anemia. Although the experimental evidence is provocative, the association of a virus with a pathological syndrome is insufficient to prove causation (Duesberg, 1987; Hill, 1965). Historically, several criteria have been employed to determine the cause of a disease. In the case of infectious diseases, Koch’s postulates have frequently been used to establish the necessary association of specific agents with specific diseases (Anderson and May,
Although now considered fallible and primarily restricted to laboratory models of disease (Hanson, 1988), Koch's postulates are still heavily relied upon to provide evidence to support causal hypotheses in the investigation of fish diseases (Chapter 1). In the case of marine anemia however, Koch’s postulates have yet to be fulfilled: It has not been established that the salmon leukemia virus is present in all cases of the disease; that the agent is not present in other diseases or normal tissues; that the organisms can be isolated from tissues in pure culture; or that the organism is capable of inducing the disease under controlled conditions, Indeed, the virus has yet to be isolated. Electron micrographs and biochemical reactions, such as reverse transcriptase activity are the basis for inferring the existence of this new virus (Eaton and Kent, 1992). Until a pathogen can be obtained in pure culture, Koch’s postulates for marine anemia will remain unfulfilled.

Experimental transmission studies have established that the lesions of marine anemia can be replicated by the injection of ultrafiltered tissue homogenates (Kent and Dawe, 1993). This has been used as evidence that the disease is infectious and caused by an organism less than 0.22 microns in size, such as a retrovirus (Kent and Dawe, 1993). However, the results of such experiments do not unequivocally indicate a viral etiology. A virus was
implicated as the cause of a "kidney tumour" of tropical fish after the lesion could be readily replicated by intraperitoneal injection of cell-free, ultra-centrifuged tissue homogenates (Beckwith and Malsberger, 1980). However, when the transmission experiments were replicated, the supposed neoplasm was instead classified as a necrotic, granulomatous lesion induced by the inadvertent injection of soluble mycobacterial cell-fractions into fish harbouring low-grade infections with *Mycobacterium fortuitum*. The association of marine anemia with a variety of infectious agents (Kent et al., 1990), and the undefined role of immune stimulants, prevents one from discarding the alternative hypothesis that the experimental replication of marine anemia may in fact be the result of transmitting cell products or other "contaminants" that stimulate an exhuberent immune response which is later manifested as marine anemia. Successive "transmission" of marine anemia in laboratory experiments would not preclude this alternative hypothesis. As retroviruses exploit actively dividing cells for their replication (Duesberg, 1987), it should not be surprising that an endogenous retrovirus can be detected at high titres in animals undergoing excessive cell division. The injection of microorganisms at high dosages and by routes that by-pass normal host defence mechanisms can allow a normally non-pathogenic organism to cause disease (Hanson, 1988). Before resources and effort
are committed to developing mass immunization programs for marine anemia, it is essential that the role of the salmon leukemia virus in marine anemia be further investigated. To do this, a reliable method of detecting and isolating the agent in natural and experimental cases of the disease is required.

As with mass immunization, the lack of an established pathogen for marine anemia prevents any specific recommendation for a chemotherapeutant for use in a mass treatment program. The British Columbia salmon farming industry is remarkably limited in the number of chemotherapeutants that are licensed for use in mass medication of salmon intended for human consumption. Currently, only three products are licensed for the treatment and control of infectious diseases: oxytetracycline and two potentiated sulphonamide products. The efficacy of these products for controlling marine anemia is unknown. However, the association of marine anemia with non-productive inflammatory diseases such as vibriosis and Enterocytozoan salmonis infections suggests that the control of these diseases by chemotherapy or chemoprophylaxis may be indicated on farms experiencing or anticipation marine anemia problems. However, as marine anemia rarely contributed to more than 6% of the total number of mortalities, and the majority of deaths on marine anemia positive farms were attributed to other infectious
diseases, benefits of mass medication programs using currently available products would likely be realized through their impact on communicable diseases other than marine anemia.

A syndrome histologically "identical" to marine anemia has been described in fresh water chinook in California (Kent, 1992; Hedrick et al, 1990). The protozoan *E. salmonis*, has been implicated as the cause of this fresh water leukemia. Although the role of the parasite in the genesis of these lesions is unclear, the mass treatment of fish with the antiprotozoal agent fumagillin has resulted in the resolution of the disease in California tank-based experiments (Hedrick et al, 1991). If the association seen between marine anemia and non-productive inflammatory diseases is shown in future research to be causal, then, as in the case of chinook leukemia in California, the reduction of concurrent infections may prove to be an effective means of reducing the incidence of marine anemia in seapens. However, until such a relationship is established, the effect of mass medication on marine anemia remains speculative.

As suggested in chapter seven, most infectious diseases are the result of an imbalance between the ecological relationship between the hosts, the agent, and the environment. If the environment can be modified to reduce the likelihood of such imbalances, the level of
disease would also be reduced (Martin et al., 1987, p.253). Mathematical models of infectious disease, such as the Reed-Frost model, demonstrate that the level of disease in a population can be manipulated by affecting the adequacy of contact with an infectious agent, and by altering the proportion of susceptibles in the population (Anderson and May, 1991). Whereas crowding, mixing, and the failure to remove early cases of disease from pens surely increase the probability of contacting a pathogen, the "abnormal" oceanographic, social, and nutritional factors created by salmon farming undoubtedly increases the susceptibility of farmed salmon to infectious disease (Peterson et al., 1991). A brief review of the life history of chinook salmon clearly illustrates the extreme distortion of chinook ecology that results from seacage culture (Groot and Margolis, 1991). In populations experiencing environmental and ecological pressures, most morbidity and mortality is due to an increase in diseases already common in the population (Shears, 1991). If the number of individuals that are susceptible to a disease can be reduced, the magnitude and duration of a disease outbreak can be diminished (Martin et al., 1987, p. 203-205). Because evidence suggests that marine anemia is an endemic disease (Chapter 2 & 7) and that the occurrence of marine anemia on a farm is associated with indices of poor health, such as elevated mortality rates and excessive losses due to a
variety of infectious agents, the modification of salmon farm environments to promote general health would be an important component of future programs developed for reducing losses on farms affected by marine anemia.

To reduce the "stresses" of captivity that predispose farmed salmon to disease, the ecology, physiology, and social structure of the chinook salmon must be considered. Wild chinook salmon are non-schooling, opportunistic feeders, that spend much of their life-cycle in environments substantially different than those encountered in near-shore seapens (Groot and Margolis, 1991). By reducing stocking densities, relocating seapens to cooler waters, and modifying feed formulation and delivery many of the stressors created by intensive salmon farming may be reduced. In addition, consistent farm-level disease surveillance, careful screening and selection of replacement stock, and the reduction of mixing of stocks in pens would help producers to avoid the introduction and propagation of infectious agents on their farms. However, the economic pressures that created many of the undesirable rearing conditions on British Columbia salmon farms continue to exist today, making it difficult to implement health promotion programs based on improving environmental and ecological conditions. The revision of fish farm management practices that would be required to affect or reduce the level of infectious disease on a salmon farm
would require a well planned supportive educational program.

Rarely have educational programs been successfully applied to disease control programs in veterinary medicine (Schwabe et al., 1977). However, this investigation of marine anemia demonstrated the need to educate fish farmers and fish health professionals in matters of salmon farm health and disease. The development of fish health education programs, which include not only instruction on the treatment of a disease, but also on such diverse areas as how to determine the health of a farm, the difference between infection and disease, the importance of promoting health as opposed to dealing with disease, and the necessity of evaluating a disease on the farm before interventions are planned, should become priorities for fish health providers and the British Columbia salmon farming industry.

There are, theoretically, three stages at which we can reduce the effect of a disease on individuals and populations: (1) prevent the disease from occurring in the first place by promoting general health or by applying specific protective measures; (2) detect the disease early so that prompt remedial actions can be undertaken to slow its progression and prevent its spread; or, (3) limit the impact and speed the recovery of those already affected by the disease (Maunser and Bahn, 1974). Based on the evidence
generated in this study, health promotion through improved environmental and management conditions on salmon farms may be the most reasonable focus for future efforts to prevent and control marine anemia.

As the British Columbia salmon farming industry has evolved, the level of concern regarding marine anemia has decreased considerably. The production of chinook salmon, once responsible for the majority of farmed salmon marketed in British Columbia, was surpassed in the 1990’s by Atlantic salmon production (Anon, 1992). As the disease is not currently perceived to be a threat to Atlantic salmon, some observers have been prompted to question the need to devote future efforts towards the development and refinement of marine anemia control and prevention programs. However, the susceptibility of Atlantic salmon to experimental replication of marine anemia (Newbound and Kent 1991b) and the finding of marine anemia-like lesions in farmed Atlantics as well as in apparently wild stocks of chinook suggest that we should not dismiss marine anemia as a disease no longer of concern. Instead, attempts should be made to synthesize new and existing information to develop potential intervention strategies not only to service the remaining chinook producers in the province, but also in preparation for the possibility of marine anemia becoming a problem for other farmed and wild species. Future efforts to discover the causes of marine anemia will not only
provide new approaches to its treatment and control, but also will undoubtedly provide new insights into carcinogenesis, immunology and the biology of seapen disease.
References


