



Fisheries and Oceans Canada

Pêches et Océans Canada

Science

Sciences

## **Canadian Science Advisory Secretariat (CSAS)**

---

**Research Document 2014/060**

**Pacific Region**

### **Monitoring for sea lice on wild salmon in western and eastern Canada**

Stewart Johnson and Simon Jones

Pacific Biological Station  
3190 Hammond Bay Road  
Nanaimo, B.C. V9T 6N7

---

## Foreword

This series documents the scientific basis for the evaluation of aquatic resources and ecosystems in Canada. As such, it addresses the issues of the day in the time frames required and the documents it contains are not intended as definitive statements on the subjects addressed but rather as progress reports on ongoing investigations.

Research documents are produced in the official language in which they are provided to the Secretariat.

### Published by:

Fisheries and Oceans Canada  
Canadian Science Advisory Secretariat  
200 Kent Street  
Ottawa ON K1A 0E6

[http://www.dfo-mpo.gc.ca/csas-sccs/  
csas-sccs@dfo-mpo.gc.ca](http://www.dfo-mpo.gc.ca/csas-sccs/csas-sccs@dfo-mpo.gc.ca)



© Her Majesty the Queen in Right of Canada, 2015  
ISSN 1919-5044

### Correct citation for this publication:

Johnson, S. C. and Jones S.R.M. 2015. Monitoring for sea lice on wild salmon in western and eastern Canada. DFO Can. Sci. Advis. Sec. Res. Doc. 2014/060. vi + 33 p+ Appendices.

---

---

## TABLE OF CONTENTS

ABSTRACT.....	V
RÉSUMÉ.....	VI
INTRODUCTION .....	1
PROGRAM DESIGN CONSIDERATIONS .....	1
SEA LICE: BIOLOGICAL, ECOLOGICAL AND BEHAVIOURAL DIFFERENCES .....	2
HOST RANGE .....	2
BIOLOGICAL AND ECOLOGICAL DIFFERENCES.....	2
BEHAVIOURAL DIFFERENCES .....	2
HOST BIOLOGY.....	3
HOST POPULATION ABUNDANCE AND GENERAL CONDITION .....	3
MIGRATION PATTERNS AND RATES OF PASSAGE OF JUVENILE SALMON .....	3
ATLANTIC SALMON .....	3
PACIFIC SALMON.....	4
Pink and Chum Salmon.....	5
Sockeye Salmon .....	5
Chinook Salmon .....	6
Coho Salmon.....	6
NON-SALMONID HOST: DISTRIBUTION AND ABUNDANCE.....	6
ATLANTIC WATERS .....	6
PACIFIC WATERS .....	6
OCEANOGRAPHIC CONDITIONS .....	7
USE OF OCEANOGRAPHIC MODELS TO AID IN STUDY DESIGN .....	7
Bay of Fundy .....	8
British Columbia .....	8
DESIGN CONSIDERATIONS FOR SAMPLING PROGRAMS.....	9
WEB BASED-TOOLS .....	9
FREQUENCY AND DURATION OF SAMPLING.....	9
SAMPLE SIZE .....	10
Status of Host Stocks .....	10
SAMPLING METHODS AND PROTOCOLS .....	11
Sampling Gears.....	11
Sampling protocols and sea lice and juvenile fish identification .....	11
SEA LICE REPORTING .....	13
HOST PARAMETERS: METHODS OF MEASUREMENT AND REPORTING .....	14

---

SEA LICE AND JUVENILE FISH IDENTIFICATION .....	16
Identification of Sea Lice .....	16
Identification of Juvenile and Adult Salmon.....	19
PROTOCOLS FOR THE MANAGEMENT, DISSEMINATION AND ANALYSIS OF DATA RESULTING FROM MONITORING PROGRAMS.....	20
REFERENCES .....	28
APPENDIX 1: STANDARDS, PROTOCOLS & GUIDELINES FOR RESEARCH INVOLVING WILD/CULTURED FISH INTERACTIONS WITH SEA LICE .....	34
APPENDIX 2 : PROTOCOLS & GUIDELINES A REFERENCE MANUAL FOR RESEARCH INVOLVING WILD/CULTURED FISH INTERACTIONS WITH SEA LICE.....	18

---

## **ABSTRACT**

Well-designed and executed, systematic surveillance programs are necessary to obtain sea lice infection data from wild fish populations. These data are necessary to inform decision makers with respect to the occurrence of sea lice parasitism within the wild population and the extent of bi-directional interactions of sea lice between the wild and farmed populations. This section builds upon the peer-reviewed “Protocols and Guidelines for the study of interactions between salmonids and sea lice” that were developed by the BC Salmon Forum in 2006 (Appendix 1). Various methods that are available for use in sea lice survey work are reviewed and an indication of their advantages and disadvantages is provided. As well, aspects of the biology of sea lice, salmon and non-salmonid hosts and the environmental factors that need to be considered when planning sea lice surveys are also reviewed. It is extremely challenging to design sea lice monitoring programs that take into consideration: ecological and behavioural differences between species of sea lice, ecological and behavioural differences between host species or hosts of different ages, the complex interactions between sea lice and salmon, the inherent natural variability of large ecosystems and the complex interactions which occur between hosts, sea lice, and environmental factors. Furthermore, it is very unlikely that a single survey design or set of methods will be optimal for all situations under which monitoring of sea lice on wild fish may be conducted. Surveys need to be designed and methods selected based on the goals of the monitoring program keeping in mind logistic and financial constraints. Limitations posed by survey design or the methods used need to be carefully considered during interpretation of data and clearly communicated during reporting.

---

## **Surveillance du pou du poisson sur le saumon sauvage dans l'Ouest et l'Est du Canada**

### **RÉSUMÉ**

Des programmes de surveillance systématiques, bien conçus et bien exécutés, sont nécessaires pour l'obtention de données sur les infections par le pou du poisson dans les populations de poissons sauvages. Ces données sont nécessaires pour informer les décideurs en ce qui concerne l'occurrence du parasitisme par le pou du poisson dans la population sauvage et l'étendue des interactions bidirectionnelles des poux du poisson entre les populations sauvages et de culture. Cette section repose sur les « Protocoles et Lignes directrices pour l'étude des interactions entre les salmonidés et le pou du poisson » qui ont été élaborés par le Forum du saumon de la C.-B. en 2006 (annexe 1). Diverses méthodes qui peuvent être utilisées dans les travaux de relevé portant sur les poux du poisson sont évaluées et une indication de leurs avantages et de leurs désavantages est fournie. De plus, on examine les aspects de la biologie du pou du poisson et des hôtes salmonidés et non salmonidés, et les facteurs environnementaux qui doivent être pris en considération lors de la planification des relevés du pou du poisson. Il est extrêmement difficile de concevoir des programmes de surveillance qui prennent en considération : les différences écologiques et biologiques entre les espèces de poux du poisson, les différences écologiques et comportementales entre les espèces hôtes et les hôtes d'âge différent, les interactions complexes entre le pou du poisson et le saumon, la variabilité naturelle inhérente aux grands écosystèmes et les interactions complexes qui ont lieu entre les hôtes, les poux du poisson et les facteurs environnementaux. En outre, il est très improbable qu'une seule conception ou un seul ensemble de méthodes de relevés soit optimal pour toutes les situations de surveillance du pou du poisson chez les saumons sauvages. Les relevés doivent être conçus et les méthodes choisies en fonction des buts du programme de surveillance en tenant compte des contraintes logistiques et financières. Les limites imposées par la conception des relevés ou les méthodes utilisées doivent être considérées soigneusement lors de l'interprétation des données et clairement communiquées au moment de rédiger les rapports.

---

## INTRODUCTION

When obtained from well-designed and executed, systematic surveillance programs, sea lice infection data obtained from wild fish populations will inform decision makers with respect to the occurrence of sea lice parasitism within the wild population and the extent of bi-directional interactions of sea lice between the wild and farmed populations and the effectiveness of sea lice management strategies applied within farmed salmon populations for reducing sea lice numbers in the vicinity of farms.

In British Columbia a large number of sampling programs have examined sea lice levels on wild salmonids (Table 2). This is in contrast to studies on wild Atlantic salmon on the east coast of Canada and Maine where only 2 studies have been reported on. These sampling programs have varied in their design and implementation dependent upon the scientific question, program objectives and available resources.

In 2006 the BC Salmon Forum produced a series of peer-reviewed Protocols and Guidelines for the study of interactions between salmonids and sea lice. In the document "Protocols and Guidelines 2 Field sampling methods for juvenile and adult Pacific salmon and caligid zooplankton," there is an extensive review of sampling of wild fish for sea lice (Anonymous, 2006, Appendix 1). In the document "Protocols & Guidelines 1 A Reference manual for research involving wild/cultured fish interactions with sea lice: sea lice biology, identification and laboratory methods," methods to identify sea lice which occur in British Columbia were provided (Galbraith et al., 2006, Appendix 2). The purpose of this section is to summarise the elements of effective surveillance programs for the collection, interpretation and dissemination of information relating to sea lice infections in wild populations.

## PROGRAM DESIGN CONSIDERATIONS

Most studies of sea lice on wild salmonids fall within the broad categories of descriptive studies and analytic studies. By definition descriptive studies, which includes surveys, examine populations and are not designed to provide for comparisons between groups and therefore do not support hypothesis testing. Analytic studies are designed to identify portions of populations which differ in their exposure to sea lice with the goal of identifying risk factors associated with infection (e.g., exposure to salmon farms).

Analytic studies can include both experimental and observational studies. Descriptive studies and observational studies differ from laboratory experimental studies as there is no assignment of individuals to experimental groups, no control over whether a particular individual is exposed to sea lice, and no control over the environmental conditions under which the exposure occurs. For this reason results obtained from such uncontrolled studies must be interpreted with caution as: groups of animals (as defined by exposure to sea lice) may differ from each other in other ways than just the exposure to sea lice; and the fact that these interactions are taking place in environments that are inherently variable.

It is extremely challenging to design sea lice sampling programs that take into consideration: ecological and behavioral differences between species of sea lice, ecological and behavioural differences between host species or hosts of different ages, the complex interactions between sea lice and salmon, the inherent natural variability of large ecosystems and the complex interactions which occur between hosts, sea lice, and environmental factors (reviewed in Anonymous, 2006). Further, many sampling programs will be constrained by logistic and financial realities which need to be carefully considered during the design process. The balance between the objectives of a program, its logistics and the cost of program delivery can only be usefully determined on a case-by-case basis (Anonymous, 2006).

---

Sampling programs need to be carefully planned with clearly defined goals and scientific objectives and limitations of the program must be clearly articulated in advance. The programs need to, as much as possible, take into consideration the biology, ecology and behaviour of the different sea lice species and their salmonid and non-salmonid hosts, as well as the physical, chemical and biological features of the environment. Researchers planning monitoring programs for sea lice, especially those designed to detect trends or to examine impact of salmon farms on sea lice, need to consider: the number of years that the study needs to be conducted, and the power of their study design to detect certain sized trends. These issues are discussed in more detail below.

## **SEA LICE: BIOLOGICAL, ECOLOGICAL AND BEHAVIOURAL DIFFERENCES**

### **HOST RANGE**

The five species of sea lice commonly found on salmonids in Canadian waters vary widely with respect to the number and species of salmonid and non-salmonid hosts on which they naturally occur (Jones and Johnson, 2013, Table 2). For example, as part of the design of a sea lice sampling program to investigate the contribution of salmon farms as sources of sea lice, the selection of sampling gear types should take into consideration whether the gear type is suitable for capturing resident salmonid and non-salmonid hosts of differing ages that may be present within the study area.

### **BIOLOGICAL AND ECOLOGICAL DIFFERENCES**

With the exception of *L. salmonis*, little is known about the biology and ecology of species of sea lice found on salmon (reviewed in Jones and Johnson, 2013). However, it is likely that all species of sea lice show similar general trends with respect to environmental conditions. For example, all species of sea lice likely have higher rates of development at higher temperatures, although the exact rates of their development and/or tolerance to extreme temperatures may differ. With respect to salinity, we can expect that all species of sea lice show reduced survival at low salinity, but again there may be marked differences between species with respect to the ability to tolerate and survive in waters of low salinity.

With respect to the interpretation of sea lice counts on wild fish, the value of knowledge of their biology and ecology is well recognized. However, how such knowledge can be applied to the design of sampling programs hasn't been formally addressed. It may be possible to use information on the physiological/environmental tolerances of sea lice to exclude regions of particular water characteristics from sampling programs. Knowledge of the rate of sea lice development and the migration rates of the wild salmon hosts could be used in part to support decisions related to selection of sites (location and number) and the frequency at which sampling occurs. These decisions would also be effected by the nature of the questions being asked.

### **BEHAVIOURAL DIFFERENCES**

It has generally been observed that *Lepeophtheirus salmonis* when compared to species of *Caligus* is less likely to leave its host upon disturbance. For all species of sea lice a proportion of copepodid, preadult and adult stages are subject to being lost during the process of host capture and processing. The proportions that are lost during capture are not known, however, general experience suggests that higher proportions of *Caligus* spp. will be lost. For field studies, sampling devices and methods have been developed to limit the number of sea lice lost during these activities. Examples include: use of fine mesh dip nets, capture of fish directly into sampling bags or individual sampling containers (Jones and Hargreaves, 2007; Butterworth et



---

al., 2008; Beamish et al., 2009) and the modification of trawls for live capture (Lacroix and Knox, 2005; Gottesfeld et al., 2009). Therefore great care must be taken to ensure that methods used to capture and sample fish are consistently applied throughout any program. Similarly, comparisons of data from among different sampling programs must take into consideration differences in sampling methods.

The ability of all species of sea lice to transfer as preadult and adults between hosts needs to be considered when discussing sea lice stage distribution on wild hosts. Estimates of transfer between hosts in the field are limited to studies on farmed salmon in which 63% of male and 52% of female sea lice transferred between hosts over a 4-day period (Ritchie, 1997). Jones and Prospero-Porta (2011 and references therein) report abundant chalimus but relatively low numbers of preadult and adult *L. salmonis* and *C. clemensi* on three-spine sticklebacks suggesting that these motile stages may leave the host. The proportion of these stages that transfer to juvenile salmon is unknown. Transfer of mobile stages from farmed and wild salmonid and non-salmonid hosts may explain reports of preadult and adult sea lice on juvenile salmon that have recently entered the marine environment. As reported in Saksida et al. (2015), *Caligus clemensi* from wild hosts is known to infect Atlantic salmon as preadults and adults.

## HOST BIOLOGY

### HOST POPULATION ABUNDANCE AND GENERAL CONDITION

Data on the abundance of different wild and farmed host species within the study area are important to provide an estimate of the size of the sea lice population and the relative potential contribution of the different host populations to observed infection levels. Data on the abundance of hosts on salmon farms can be obtained. Obtaining accurate estimates of abundance of wild fish is problematic as many gear types used in sea lice surveys will only allow for the estimation of relative abundance (e.g., catch per unit effort (CPUE); Table 2). Trawling is the best method for determining the abundance of host species assuming the catch efficiency of the net is known. Unfortunately, trawling is a poor method for collecting fish for sea lice enumeration due to high levels of host abrasion with loss of scales and sea lice which occur during capture.

The general condition and age-structure of salmonid host populations are also important. To date most sea lice surveys on wild fish have focused on hosts that have recently entered seawater as these early marine residents are believed to be the most vulnerable to sea lice infections due to their small size and high energy requirements to sustain their growth. The collection and reporting of basic host data including host length and weight is necessary as these data may be used to estimate risk associated with infection, as well as provide an estimate of host age or time spent in seawater.

### MIGRATION PATTERNS AND RATES OF PASSAGE OF JUVENILE SALMON

#### ATLANTIC SALMON

Many studies have examined Atlantic salmon movements and mortality in coastal waters in regions where there are now salmon farms. With respect to the Bay of Fundy, studies conducted up to 2004 have been reviewed in Dadswell (2004). More recently, Lacroix and Knox (2005) examined the distribution of postsmolt Atlantic salmon from different origins in the Bay of Fundy over the period of 2001 to 2003. They reported that distribution of both wild and hatchery fish within the Bay of Fundy reflected the major surface-current vectors with fish being

---

aggregated in some areas. As fish moved into the outer bay and into the Gulf of Maine they became more dispersed. In another study Lacroix et al. (2005) reported on migratory route, rate of migration and survival of Atlantic salmon from the Big Salmon River based on telemetry data collected in 1999. Telemetry data obtained for juvenile Atlantic salmon originating from three regions in the inner Bay of Fundy has also been used to assess stock specific migration patterns and migration success (Lacroix, 2008). Data on the distribution of Atlantic salmon in near shore of the Gulf of Maine are also available for the years 2001 through 2005 (Sheehan et al., 2011). These and earlier studies have been summarized in the Maritime Region Canadian Science Advisory Secretariat Science Response 2011/001 entitled “Wild salmon populations in the vicinity of a proposed finfish aquaculture development in St. Mary’s Bay, Nova Scotia” (DFO, 2011).

Limited data from a small number of mark-recapture studies, historical commercial fisheries and low spatial resolution satellite tagging studies have been used to assess the distribution and residence times of adult Atlantic salmon in the Bay of Fundy (reviewed in DFO, 2011). These data don’t have the spatial and temporal resolution necessary for planning of field-based sampling programs.

In Newfoundland many Atlantic salmon farms are located within Bay D’Espoir. Recently, the migration routes and survival of Atlantic salmon smolts have been reported on for Bay D’Espoir based on data collected using acoustic tags over the period of 2006 to 2008 (Dempson et al., 2011). These authors reported different migration routes for fish from the Conne and Little Rivers. Data on the residency of smolts within different zones of the estuary are given, as well as survival estimates for these two river populations.

Although not in an area of salmon farming, post-smolt migration routes have also been investigated using acoustic tagging for salmon from the Rivière Saint-Jean which is located on the north shore of the Gulf of Saint Lawrence (Lefevre et al., 2012). Data are presented for 2009 and 2010 and the authors provide information on directions of movement and influence of environment factors on post-smolt migration.

Taken together there is sufficient information on post-smolt Atlantic salmon movement in Eastern Canada to support the planning of wild fish sea lice surveys in the majority of areas that are presently used for salmon farming. Interestingly this is not true for most species of Pacific salmon and regions of British Columbia.

## **PACIFIC SALMON**

In comparison to the Atlantic salmon remarkably little is known about patterns of migration, residence time within inshore waters and run timing for most Pacific salmon stocks in British Columbia. There are several reasons for this including the: low economic value of some species (pink and chum), large number of systems of origin, vast geographical area they inhabit and the long held belief that production was controlled by factors within the freshwater and by mortality later in the life cycle including mortality from fishing. A review of data related to early marine life of Pacific salmon which includes some information on migration and residency times is presented in Beamish et al. (2003). For the purposes of this section the focus will be on information that is available for the major salmon farming areas in BC.

With respect to the near shore waters, different species, stocks and life history types appear to have specific migration routes however these have not been well defined. It is possible that migration routes and residency times may not be consistent over the period of migration and/or between years. Sources of information for juvenile salmon are provided below. In addition, DFO Program for Aquaculture Regulatory Research (PARR) supported sea lice surveys conducted in the Strait of Georgia and Johnston Strait from 2010-2012 are providing data on the

---

distribution and relative abundance of juvenile salmonid and non-salmonid species in these areas. These data have not yet been analyzed.

### **Pink and Chum Salmon**

Although abundant in many areas of BC, the migratory routes, timing of sea water entry, and migration rates of pink and chum salmon are essentially unknown. In the case of the Broughton Archipelago there has been a great deal of debate with respect to the migratory routes and residence time of pink and chum salmon in this geographically complex area. Some researchers have “presumed” that there is a “main migration corridor” out through Tribune Channel and Fife but there are no data to support their presumption (Morton et al., 2005; Krkosek et al., 2006). Collections of juvenile pink and chum salmon made in 2003 found these species to be widely distributed throughout the Broughton and Knight Inlets with similar abundances in Knight Inlet, Wells Passage, Fife Sound and Tribune Channel (Hargreaves unpublished). Hargreaves (unpublished data) felt that these data do not support the view of a “main migration corridor”. However, he goes on to note that under different conditions and fish abundances, different patterns of fish distribution and migration may occur. The assessment of pink and chum migration routes within the Broughton Archipelago using the large amount of data collected during annual sea lice surveys from 2003 to date will be limited by the purse and beach seine gear used to collect these samples which are not well-suited for the estimation of stock abundance (Table 2).

There is only general information on the migration patterns and residency time of pink and chum salmon in other near shore areas. During even years, a large proportion of juvenile pink salmon in the Strait of Georgia are of Fraser River origin, although smaller watersheds, especially along the northern shores of the Strait also produce pink salmon. Many stocks of pink salmon enter in the early spring (March-April) and remain initially close to shore, migrating to deeper waters as they grow. Abundance and distribution data support the belief that the majority of pink salmon leave the Strait by late July. The residence times for pink and chum salmon in areas of salmon farming is not known but is likely to be highly variable depending on whether they are of Fraser River origin or originating from smaller systems that are near to salmon farming areas.

### **Sockeye Salmon**

The distribution of juvenile sockeye salmon within in the Strait of Georgia was first reported by Groot et al. (1985) using data collected in the springs of 1982 to 1985. Using these data Groot and Cooke (1987) proposed a migratory route for the majority of stocks of Fraser River sockeye salmon through the Strait of Georgia. Based on more recent data their proposed migratory route seems to be generally correct for most stocks of juvenile sockeye. Exceptions to this may include Lower Fraser River stocks such as Harrison Lake which: migrate to sea later in the year, may have a longer period of residency within the Strait of Georgia and may migrate to the open ocean through the Strait of Juan de Fuca rather than travelling northward through the Strait of Georgia. However, recent observations of Harrison Lake sockeye in near shore waters of British Columbia suggest that their migration pathway needs to be reassessed (Marc Trudel pers. comm.). There are limited data on the distribution of juvenile sockeye in other areas of BC. Bi-weekly surveys were conducted from May 1 to June 30, 1998 in Barkley Sound. Although these data were not published they are available in a contract report (Groot, 2011).

Previous estimates of the residence times for juvenile Fraser River sockeye salmon in the Strait of Georgia ranged from 14 to 38 days (reviewed in Preikshot et al., 2012). Based on their recent analysis, Preikshot et al. (2012) estimated a longer average residence time, ranging between 43 and 54 days.

---

## **Chinook Salmon**

Understanding the migration patterns of chinook salmon is complicated by differences in timing of migration. Chinook salmon are classified as ocean-type (migrate to sea within the first year) and stream-type (migrate to sea after a full year in freshwater). Both types enter the ocean starting in April – May, with the stream type remaining resident within the Strait of Georgia for about 2 months and the ocean-type for one to two years following sea water entry. The majority of Fraser River chinook salmon are thought to travel northwards through the Strait of Georgia and Johnstone Strait with a smaller proportion leaving through Juan de Fuca Strait (reviewed in Beamish et al., 2003; Melnychuk et al., 2010). Interestingly in recent years the historical pattern of migration with respect to time has apparently shifted. Most recent studies suggest that populations of both stream- and ocean-type fish remained in the Strait of Georgia through to mid-September, each type showing a different depth preferences (Beamish et al., 2011). There are likely differences between populations with respect to their distribution in the environment. For example the Cowichan River population was reported to rear primarily in the area of their natal river up to the point at which they migrated (Beamish et al., 2011). There are no data on migrations of juvenile chinook salmon in other near shore areas of British Columbia.

## **Coho Salmon**

Based on acoustic tagging studies, juvenile coho salmon are reported to spend a several months within the Strait of Georgia with the majority believed to leave in September to October of their first year (Chittenden et al., 2009). Detailed routes of migration have not been determined for the Strait of Georgia. In addition there are no data on migrations of juvenile coho salmon in other near shore areas of British Columbia.

As evidenced by the above, we have a general knowledge of the large scale movements of salmon with the near shore environment and a basic understanding of their residence time within some areas. However, the resolution of these data is at a large scale and specific migration routes on the smaller scale remain unknown. It is also unknown as to whether migration routes and residence times will be consistent both within and between years as there may be many environmental factors that influence them. To develop data on specific migration routes would requires the use of acoustic or other forms of mark-recapture studies conducted over long periods of time. Genotyping of individuals to determine stock of origin is also required to support such studies. At this time funding for such large scale programs is not available.

## **NON-SALMONID HOST: DISTRIBUTION AND ABUNDANCE**

### **ATLANTIC WATERS**

Using multiple years of data from research trawl surveys and the World Wildlife Fund seascape approach, the distribution and relative abundance of cod, pollock and winter flounder within the Bay of Fundy has been mapped (Bredin et al., 2004). Additional information on non-salmonid hosts distribution is available for the southern Bay of Fundy and south eastern Nova Scotia at the Gulf of Maine Area, [Census of Marine Life website](#).

### **PACIFIC WATERS**

With the exception of the Pacific herring, there is little known about the distribution of non-salmonid hosts of sea lice within the inshore waters off British Columbia. For the Broughton Archipelago capture data for non-salmonids obtained as part of sea lice survey work (2003 – present) contains some information about the distribution and relative abundance of some non-salmonid hosts of sea lice. In addition the 2010-2012 DFO PARR study of the Strait of Georgia

---

and Johnston Strait provides some information on the distribution and relative abundance of non-salmonid hosts such as herring and three-spine stickleback.

At this time information about the distribution, migration and residency time of salmonid and non-salmonid hosts in near-shore BC, Atlantic Canada and Newfoundland waters lacks sufficient detail to be of much use in planning wild salmon sea lice monitoring programs. Non-salmonid hosts do need to be considered with respect to the selection of sampling gear to ensure that if they are present they will be represented in the samples. With respect to herring and sticklebacks the gear types that are commonly used (e.g., beach seines, modified purse seines) to catch juvenile wild salmonids will capture these hosts provided small enough mesh sizes are used. However, there are numerous other non-salmonid hosts that such gear types will not catch.

With respect to the identification of the sources of sea lice counts found on wild fish, numerous authors have identified the importance of understanding the numbers of non-salmonid hosts and the numbers of sea lice that they carry. This is especially important in some areas of BC where non-salmonid hosts can be very abundant and heavily infected with sea lice (Jones et al., 2006). For some species such as the three-spine stickleback it has been suggested that they may serve as useful sentinel species for sea lice (Jones et al., 2006; Beamish et al., 2009; Jones and Proserpi-Porta, 2011)

## **OCEANOGRAPHIC CONDITIONS**

As reported in Jones and Johnson (2014), environmental factors such as temperature and salinity have marked effects on the development, survival and infectivity of sea lice. In addition the free moving stages of sea lice have (naupliar, copepodid, preadult and adult) have limited swimming capability so their distribution especially in the near shore environment will be effected by prevailing tides and currents (Brooks, 2005; Stucchi et al., 2011).

When designing sampling plans, especially those designed to study sea lice distribution in within the environment, site specific oceanographic conditions must receive careful consideration (reviewed in Brooks, 2005). Consideration of specific oceanographic conditions will support the interpretation of data and help prevent situations where the use of particular sites as either “control” or sites outside of “salmon farm zones of infection” have been questioned based on differences in their oceanographic conditions, as well as presence/absence of other host species (e.g., Price et al., 2010; Jones and Beamish, 2012; Price and Reynolds, 2012). At present the use of oceanographic conditions in the planning of surveys is limited to studies focusing on *L. salmonis* as this is the only species for which we have data on its environmental tolerances (see Jones and Johnson, 2014). Such data are not available for *L. cuneifer* and *Caligus* species.

## **USE OF OCEANOGRAPHIC MODELS TO AID IN STUDY DESIGN**

The free swimming stages of sea lice are relatively poor swimmers when compared to free-living crustacean zooplankton which means that physical oceanographic processes will have a significant effect on how they are distributed within the environment. Oceanographic circulation models once validated have the potential to improve our ability to predict sea lice distributions especially in near shore areas that are subjected to strong currents. Oceanographic models have been developed on both the West and East Coast of Canada that cover some of the areas in which aquaculture occurs. These efforts are described in Page et al. (2013) and are briefly reported below.

---

## Bay of Fundy

The large-scale circulation patterns of the Bay of Fundy and adjacent waters have been studied for decades and numerous models have been developed (reviewed in Aretxabaleta et al., 2011). General circulation patterns within the Bay of Fundy are primarily driven by tides which through their interactions with bottom topography and the earth's rotation (tidal rectification) results in inflow along the Nova Scotia shelf, outflow along the coast of New Brunswick and Grand Manan Island and the establishment of a persistent gyre near the mouth of the bay (Aretxabaleta et al., 2009; Aretxabaleta et al., 2011 and references therein). In addition to these studies, modeling of water circulation on smaller scales has been completed for several Bay Management Areas (BMAs). These smaller scale modeling efforts have been used to examine the spread of infectious salmon anemia virus and aquaculture therapeutants (Page et al., 2005; Chang et al., 2007; DFO, 2013).

There is a model of coastal circulation being developed for Southern Newfoundland including Bay D'Espoir (Ratsimandresy et al., 2012).

## British Columbia

There are numerous models of ocean circulation for coastal waters of British Columbia but most of these models have spatial resolution that is much too large to make them useful in the planning of sea lice monitoring and in the interpretation of the results (see Page et al., 2013). The Broughton Archipelago and the Discovery Island Area are two near shore sites for which models that have small-scale spatial resolution have been developed.

In the Broughton Archipelago simple circulation models have been used to examine the advection of free-swimming stages of *L. salmonis* from salmon farms (Brooks, 2005; Krkosek et al., 2005a; Brooks and Stucchi, 2006). These include the use of a basic advection diffusion model by Krkosek and co-authors and the use by Brooks of a Finite Volume Coastal Ocean Model (FVCOM) as described in Foreman et al. (2006). More recently an improved circulation model for the Broughton Archipelago has been developed making this region one of the best modeled near shore regions in British Columbia (Foreman et al., 2009; Page et al., 2013). This model has been combined with biological models of: *L. salmonis* egg production and *L. salmonis* development, behaviour and survival to predict the spatial and temporal distribution of *L. salmonis* copepodids in Broughton Archipelago surface waters in 2008 (Stucchi et al., 2011). The model predictions when compared to plankton and sea lice data that were collected over the simulation period showed that the model predicted lower than observed planktonic copepod concentrations, and low copepodid abundance in areas where wild salmon carried no lice.

Recently, Foreman et al. (2012) modeled circulation patterns in the Discovery Islands using a FVCOM simulation and oceanographic data from April 2010. The model covers the region north of Texada Island to the middle of Johnstone Strait and covers a major BC salmon farming area within the Discovery Islands. Although this model was good at predicting some oceanographic features, there were problems with its prediction of currents at different levels within the water column.

Oceanographic conditions within the near shore environment are strongly influence by local conditions, especially river flows and winds which can vary widely between years. To develop an understanding of general patterns of water circulation at small scales these models would have to be run using environmental data from many years, or for a range of environmental scenarios. This means that although small-scale resolution oceanographic models can be used to assist in the development of management programs for sea lice and as an aid to interpret the results of sea lice surveys, their use in the planning of sea lice surveys is at present limited. At present the computer resources and funding necessary to improve the existing models, apply

---

them to other near shore regions and to generate general patterns of water circulation are not available.

## DESIGN CONSIDERATIONS FOR SAMPLING PROGRAMS

### WEB BASED-TOOLS

To date few if any sea lice surveys have used epidemiological tools to assist in survey planning. That said, there are a large number of web-based tools that can be used to assist in the design of sea lice sampling programs and for the analysis of sea lice data. A starting point is the [WikiVet™ website](#). This website provides an overview of the general concepts of veterinary epidemiology and a variety of links to web-based [epidemiological software programs](#) including software to assist in sample and survey design. Other sites include: [OpenEpi](#) which provides access to open source epidemiological statistical software, and [EPI-tools](#) that provides a number of calculators to assist in sampling program planning and design. [Quantitative Parasitology 3.0](#) is a freeware software package that is designed specifically for the analysis of parasite data. This software package provides: methods to calculate basic parasitological measures such as prevalence, mean intensity, etc., and methods to analyze parasite data which take into consideration the right skewed nature of parasite distributions within host populations.

### FREQUENCY AND DURATION OF SAMPLING

The nature of the scientific or management question that the surveillance effort seeks to address should be the major factor which determines the frequency and duration of sampling, as well as location of the study. However, in many instances the frequency and duration of sampling is in at least part set by other factors including: past experience, available budget, availability of equipment and/or personnel and other logistic considerations.

Ideally, sampling for surveillance (descriptive and observational studies) should be carried out at a frequency and for a duration that reflects the biology of the infection (host and parasite). The following aspects of the biology of salmon need to be considered:

- Timing and duration of migration (smolt and/or adult) which is dependent on species and in some cases stock of salmon and it is affected environmental conditions.
- Residency time of fish within the study area, which is again dependent on species and in some cases stock of salmon, as well as environmental conditions.
- Host factors such as growth rates, overall health, parasite rejection rates, etc.

The following aspects of the biology of sea lice need to be considered for each species of sea lice:

- Natural seasonal and yearly fluctuations in abundance (wild and farmed sources).
- Infectivity, virulence and infection pressure: when these aspects are high more frequent sampling needs to be considered.
- Infectivity – ability to infect hosts, related to parasite and host condition.
- Virulence - related to the degree of host damage and the possibility that the infection will result in morbidity or mortalities.
- Infection pressure: number of infectious stages present within the environment of interest.

The following aspects of the environment need to be considered:

- Changes in the physical characteristics of water masses (e.g., temperature and salinity), as noted above these will have effects on both the hosts and sea lice. For example, at high temperatures sea lice development rates are higher, host resistance may be lower,

---

etc. and increased frequency of sampling may be required to adequately monitor sea lice population changes.

## **SAMPLE SIZE**

The main purpose of calculating sample sizes during the planning stages of a study is to ensure that the study will be of an appropriate size to ensure that statistically significant differences, if they exist, can be recognized. However, this activity can also be beneficial to understand the amount of resources needed to complete the study and to provide an indication if the study can be conducted given available resources. The selection of sample size should, where possible, match the types of statistical analysis which are appropriate for the study. Sample size calculations require a variety of inputs including host population size, expected prevalence of sea lice and the confidence limits that are acceptable to the researcher. As described above there are numerous on-line programs that enable researchers to calculate sample size.

The number of fish retained during sea lice surveys and the number of fish eventually analyzed (sample size) is an important decision for researchers. Budgets, resources and logistics need to factor into this decision, as well as the level of accuracy and precision that is required to address the question at hand. Unfortunately, sea lice like many other parasites are unequally distributed in host populations with some hosts harbouring many and most harbouring few or none. Furthermore the goal of many studies is to identify small changes in sea lice numbers within a host population due to factors such as salmon farming. Taken together this requires accuracy and precision of estimates of prevalence (proportion infected), mean intensity (parasites per infected individual) and mean abundance (parasites per individuals examined) measurements that are strongly influenced by sample size.

It is important, wherever possible, that approximately equal numbers of hosts are sampled from each host species, host demographic group (e.g., age), and sampling unit (e.g., location, date, etc.). This strategy overcomes some problems that can be associated with highly aggregated sea lice distributions within host populations. For example, the highly aggregated nature of sea lice (abundance curve skewed strongly to the left) means that as sample size decreases there is a greater probability that prevalence, abundance and intensity will be underestimated. This issue makes the comparison of sea lice abundance between sites that have large differences in sample size problematic at best. In situations where this cannot be avoided researchers need to consider this issue carefully when interpreting their data.

## **Status of Host Stocks**

Sampling programs for sea lice often result in large numbers of fish being caught from which individuals are selected for destructive sampling. In addition, stress associated with fish capture may negatively impact those fish that are not collected for sea lice enumeration. Although this type of sampling may be acceptable for many species and/or stocks there are situations where stressful and destructive sampling cannot occur. A good example of such a situation is the enumeration of sea lice on wild Atlantic salmon; many stocks of which are threatened or endangered (Powell et al., 1999; Lacroix and Knox, 2005). Methods which allow the capture and enumeration of sea lice and collection of morphometric data with limited harm to the host have been developed. Such methods include the use modified trawls patterned after the design by Holst and MacDonald (2000) and the use of anesthetic baths. Krkosek et al. (2005b) report on a non-lethal method for the examination of sea lice on juvenile pink salmon. This method was shown to under detect earlier developmental stages (copepodid and chalimus) leading to underestimations of sea lice abundance when compared to lethal sampling. Furthermore species identification, especially of the copepodid and chalimus stages was not possible. In situations where non-lethal sampling is required researchers need to recognize and



---

communicate the limitations of their methods with respect to accuracy and the potential impact on the conclusions. In situations of threatened or endangered populations surveys should be carefully planned to ensure that the minimum number of fish necessary to provide a robust assessment sea lice abundance are collected.

## **SAMPLING METHODS AND PROTOCOLS**

The accuracy of sea lice counts and the ability to obtain unbiased estimates of sea lice population abundance is strongly affected by the way samples are collected, held prior to examination, and examined.

### **Sampling Gears**

In 2006, the BC Salmon Forum produced a series of peer-reviewed Protocols and Guidelines for the study of interactions between salmonids and sea lice. In the document “Protocols and Guidelines 2 Field sampling methods for juvenile and adult Pacific salmon and caligid zooplankton” (Anonymous, 2006), there is an extensive review of sampling gears. This review includes information on the personnel and vessel resources required to use these gears and the pros and cons associated with their use in sea lice studies (see Table 2). This document is provided as Appendix 1.

In Canada, sea lice assessments on wild salmon have been conducted using fish that have been caught by many types of gear (Tables 2 and 3). Unfortunately, there is no gear type that is suitable for sampling wild salmon throughout the marine phase of their lifecycle. In addition as mentioned above many gear types may not be suitable for the collection of non-salmonid hosts. When selecting gear type the species of salmon (especially as related to size and changes in their horizontal and vertical distribution in water column) and the physical conditions (shore topography, currents) of the study site need to be considered. If possible gear types that will adequately sample non-salmonid hosts should be selected. Costs and the availability of vessels suitable for deployment of the gear also must be considered. It needs to be kept in mind that different gear types will differentially sample wild fish populations depending on how they are spatially distributed in the environment. In short there is no single optimal gear for sampling wild salmon and non-salmonid hosts. In cases where salmon stocks are endangered, choices of gear type are limited to those that favour high survival of released individuals (see below). Regardless of gear type that is selected, researchers must recognize and report on the limitations of the gear type and carefully consider these limitations when using and reporting on their data.

### **Sampling protocols and sea lice and juvenile fish identification**

The removal of individual fish from the gear, their subsequent storage and the method by which they are processed also possess risks with respect to sea lice transfer and loss. Different protocols to reduce loss of sea lice during the isolation of individual hosts have been used in Canadian sea lice surveys. These protocols and examples of projects that have used them are described briefly below:

1. Capture of fish directly from the gear into individual sample bags and dewatering of the bags by pricking them with a needle, followed by freezing. This technique has been used during DFO sea lice surveys in the Broughton Archipelago and the Strait of Georgia (Jones and Hargreaves, 2007; Jones and Prospero-Porta, 2011; Saksida et al., 2012). This method is still being used in ongoing monitoring activities within the Broughton Archipelago.

- 
2. Use of fine mesh dipnets to transfer individual fish from gear into sample containers (Morton et al., 2005; Morton et al., 2008; Beamish et al., 2009).
  3. Isolation of a group of fish upon capture into plastic containers then transfer of individuals by dipnet to sample containers (Peet, 2007; Butterworth et al., 2008; Gottesfeld et al., 2008; Anonymous, 2009; Gottesfeld et al., 2009; Price et al., 2010; Price et al., 2011).
  4. Isolation of a group of fish upon capture into plastic containers then transfer of individuals into a water filled bag for examination (Krkosek et al., 2005b; Krkosek et al., 2006; Price et al., 2010; Morton et al., 2011).
  5. Isolation of individual fish immediately upon capture into plastic tubs or large plastic bags, fish removed and the containers examined for sea lice. This method has been used for gill net and troll caught samples (Johnson et al., 1996; Beamish et al., 2005a; Beamish et al., 2005b).

Most field-based monitoring programs for sea lice generate large numbers of samples, which take considerable time to examine for sea lice. In addition it can be very hard to distinguish between species of juvenile Pacific salmon in the field. Although it is preferable to examine fish while they are fresh and the sea lice alive, this often cannot be done due to insufficient resources in the field to complete sea lice counts and host species identification. The following protocols have been used to examine fish for sea lice:

1. Examination of live fish held in plastic bags followed by their release (e.g., Krkosek et al., 2006; Price et al., 2010; Morton et al., 2011; Price et al., 2011).
2. Examination of fresh samples in the field followed by laboratory confirmation of field counts and species identifications on specimens preserved in the field (especially for copepodid and chalimus stages) (e.g., Beamish et al., 2005c; Beamish et al., 2007; Butterworth et al., 2008; Beamish et al., 2009; Saksida et al., 2012).
3. Freezing of individuals immediately upon capture, shipped frozen and stored at -20C or -80C prior to laboratory analysis of sea lice numbers and confirmation of host species (e.g., Jones and Hargreaves, 2007; Gottesfeld et al., 2009; Jones and Prospero-Porta, 2011; Saksida et al., 2011). This method is also being used in the DFO PARR Strait of Georgia Sea Lice Program, ongoing studies in the Broughton Archipelago.
4. Storage of individuals upon capture on ice, shipped on ice and frozen (-20C) in the laboratory and stored prior to analysis (e.g., Johnson et al., 1996; Morton and Williams, 2003; Morton et al., 2004; Morton et al., 2005; Morton et al., 2008; Anonymous, 2009).

Fish are commonly killed by a lethal dose of the anesthetic tricaine methanesulfonate (MS222) at a concentration of at least 0.1 g/L water. Any equipment that has been used to sample the fish and the anesthetic bath if used must be carefully examined for sea lice that may have become dislodged.

It is possible to fix whole fish in the field in formaldehyde or ethanol for transport and storage but this has not been done. Most sea lice surveys have used freezing which is quick, easy and without health risks for laboratory personnel. However, the identification and staging of sea lice from frozen samples is more difficult as there may be deterioration of the samples during the process of freezing and defrosting. Once defrosted fish samples should not be held for prolonged periods at room temperature as sea lice will rapidly deteriorate. Sea lice obtained from frozen samples should be fixed rapidly in buffered formalin (5 to 10%) or 70% ethanol for long-term storage.

---

Very small fish can be examined without dissection. For larger fish, the fins, opercula and gills may need to be dissected and examined separately prior to examining the remainder of the fish. As early developmental stages of sea lice are very small it is necessary to examine all of the body parts of the fish under a dissecting microscope or with a hand held lens to obtain accurate counts. Staging of juvenile sea lice requires the use of a dissecting microscope and in some cases where appendages need to be examined in detail a compound microscope.

It is recommended for each species of sea lice that copepod numbers on the gills, fins and other body surfaces should be recorded separately as the physiological and/or immunological effects of the parasite will vary with body region (e.g., chalimus larvae on the tips of fins have a lower impact than those living on the body or gills).

## SEA LICE REPORTING

As a minimum, sea lice need to be identified to species and identified to general stage (copepodid, chalimus, preadult and adult). For studies that are designed to identify sources of sea lice on wild fish all sea lice need to be accurately identified with respect to species and developmental stage. Identification between the different developmental stages of chalimus larvae and preadults within and between species can be difficult and should be done in the laboratory using the procedures outlined below. If necessary sub-samples of sea lice for staging can be taken and fixed for later identification, however, the process by which sea lice were selected for staging needs to be clearly communicated.

A variety of terms can be used when describing sea lice numbers and their distribution on individual hosts and within host populations. The following are the most widely accepted definitions of these terms. The definitions and their use as quoted from Bush et al. (1997) are as follows:

1. **Prevalence** is “the number of hosts infected with 1 or more of a particular parasite species (or taxonomic group) divided by the number of hosts examined for that species”. Prevalence is commonly expressed as a percentage when used descriptively or as a proportion when used in mathematical models.
2. **Density** is “the number of individuals of a particular parasite species in a measured sampling unit taken from a host or a habitat”. It is recommended that density be used when an accurate census of all parasites is difficult or impossible to make. When the host is used as a sampling unit the terms intensity and abundance should be used.
3. **Intensity** (of infection) is “the number of individuals of a particular parasite species in or on a single infected host”. **Mean intensity** is “the average intensity of a particular species of parasite among the infected members of a particular host species. In other words, it is the total number of parasites of a particular parasite species found in a sample divided by the number of **hosts infected** with that parasite”.
4. **Abundance** is the “number of individuals of a particular parasite in/on a single host regardless of whether or not the host is infected”. **Mean abundance** is “the average abundance of a parasite species among all members of a particular host population”. In other words it “is the total number of individuals of a particular parasite species in a sample of a particular host species divided by the total number of hosts of that species examined”. For clarity it is recommended also that mode be reported with the mean abundance (Alan Donald, pers. comm.). **Mode of abundance** is the abundance of a parasite species which appears most frequently among all members of a particular host population. The mode is significant in describing the infection of a population as often most individuals in a population may be infected at low levels, but only a couple of individuals may have very high infection levels, which would drive up the mean

---

abundance value. Reporting the mode therefore provides a more representative description of the parasite numbers in the population.

In order to compare infection levels between hosts of different sizes a number of methods have been developed. These range from correcting the number of copepods to a standard body length or weight to calculation of body surface area and reporting sea lice numbers per unit of body surface area “lice infection density” (see Heuch et al., 2003). Body surface area can be determined using both image analysis and a model for body surface area: Body Surface Area ( $\text{cm}^2$ ) =  $a(W)^b$ , where  $a=12.05$ ,  $W$ = fish weight in grams, and  $b=0.61$  (see Glover et al., 2004; Tucker et al., 2002). This equation has been used for both Atlantic salmon and brown trout. Other authors have used the same equation with slightly different values for  $a$  and  $b$ . The actual value used does not really matter unless comparisons between different data sets are desired.

It is common in laboratory studies to report sea lice numbers in relation to host size. However, this form of reporting in field based studies is uncommon (e.g., Morton and Williams, 2003). The question arises as to whether such a measure is biologically relevant, especially when comparing between species of salmon needs to be further investigated especially since differences between salmon species in their susceptibility and rates of shedding of sea lice have been documented.

## HOST PARAMETERS: METHODS OF MEASUREMENT AND REPORTING

Physical characteristics such as length, body depth and wet weight should be obtained for each host examined. A variety of measurements are used to describe host length. These include: fork length: measured from the tip of the jaw or tip of the snout with closed mouth to the center of the fork in the tail (caudal fin); total length: measured from the most forward point of the head, with the mouth closed, to the farthest tip of the caudal fin; postorbital hypural (POH) length: measured from the hind margin of the socket of the eye to the end of the caudal peduncle and standard length: measured from the most anterior tip of the body to the posterior end of the vertebral column. Fork length is the most common length reported in sea lice studies. POH may be useful when reporting sea lice numbers from mature fish as it overcomes problems associated with modification of snout region (especially in males) at maturity and losses of caudal fin tissue.

Damage of the host including damage caused by sea lice should be noted (e.g., missing fins). Signs of other diseases should also be noted. Examination of fish for damage and the presence of disease are most accurate when fish have not been frozen or preserved in some other manner.

Ranking systems have been developed and used to describe gross lesions on adult Pacific salmon caused by preadult and adult sea lice. Johnson et al. (1996) used a ranking system to classify gross sea lice lesions on different body regions of freshly caught adult sockeye salmon. Their ranking system is as follows:

*Table 1a. Position: Posterior to dorsal fin, base of dorsal fin, and head.*

Rank	Description
0	Unmarked
1	Grazing evident; discolouration of skin surface and in some cases mild descaling and hemorrhaging
2	Early white lesion; Partial removal of epidermis and scales, resulting in patches of grayish to whitish necrotic tissue.

Rank	Description
3	White lesion; Epidermis and scales removed, lesions covered with continuous whitish necrotic tissue, no breaks in skin exposing musculature.
4	Early open lesion; White lesion with small (1-2 mm) point lesions open to musculature
5	Open lesion; Epidermis and dermis removed exposing musculature

Table 1b. Position: Perianal region

Rank	Description
0	Unmarked
1	Grazing evident; Characterized by the discolouration of skin surface, and in some cases blood seepage from the scale pockets
2	Mild descaling and hemorrhaging
3	Extensive discolouration, descaling and hemorrhaging
4	Early Open: Extensive descaling and hemorrhaging with small point lesions (1-2 mm) open to the musculature
5	Open lesion; Epidermis and dermis removed exposing musculature

More recently, a simpler ranking system has been used to describe gross lesions caused by sea lice on freshly caught adult Pacific salmon (Beamish et al., 2005c). Their ranking system is as follows:

Table 1c. Rankig system to describe lesions caused by sea lice.

Rank	Description
0	No skin damage and no red discoloration of skin surface from hemorrhaging
1	Minor red discoloration from hemorrhaging, but reduced in intensity and area; no scale abrasion but pin hole penetrations may be present
2	Moderate hemorrhaging resulting in more red color over an area about one half the size of the anal fin, minor scale abrasion may be present
3	Severe hemorrhaging, area of hemorrhaging approximately the size of the anal fin or larger and almost uniformly red; no lesions; scale abrasion common, but skin intact
4	Lesions present, skin removed and muscle exposed or skin partially removed exposing necrotic tissue; hemorrhaging at margins of lesions

There are few descriptions of gross lesions caused by sea lice on juvenile salmon and no field based studies have attempted to quantify damage caused by sea lice. Although difficult to assess, especially for earlier developmental stages of sea lice, it is feasible that such a ranking system could be developed. To develop such a system requires that the criteria for differentiating the ranks be well documented and examples of the different ranks provided.

---

## SEA LICE AND JUVENILE FISH IDENTIFICATION

### Identification of Sea Lice

Sea lice are identified to species, developmental stage and gender based on morphological features such as overall body shape and appendage structure. Features such as size, colour, position on host, and species of host cannot be used to differentiate species of sea lice. Kabata (1988) produced morphological taxonomic keys that permit the specific identification of adult male and female copepods belonging to *Caligus* spp. and *Lepeophtheirus* spp. that occur on fishes in Canadian waters. These include *C. coryphaenae*, *C. elongatus*, *C. clemensi*, *C. curtus*, *C. macarovi*, *L. bifidus*, *L. hippoglossi*, *L. nordmanni*, *L. parviventris*, *L. paulus*, *L. breviventris*, *L. parvicurris*, *L. pravipes*, *L. nanaimoensis* (male only), *L. hospitalis* and *L. oblitus*. *Lepeophtheirus cuneifer* is not included in the 1988 key because at the time it was developed this species was only known from fishes in Alaska. The parasite has since been reported from farmed rainbow trout and Atlantic salmon and from three-spine stickleback and herring off the west coast of Canada (Johnson and Albright, 1991b; Jones and Prosperi-Porta, 2011). Of the sea lice species affecting salmonids in British Columbia, *C. clemensi* was originally described by Parker and Margolis (1964) with subsequent descriptions, including earlier developmental stages, by Kabata (1972). Similarly, adult stages of *L. salmonis* were described by Kabata (1973, 1979) whereas earlier developmental stages were described later (Johnson and Albright 1991a; Schram 1993; Schram, 2004; Galbraith 2004). The adult stages of *L. cuneifer* were described by Kabata (1974).

Summarizing the taxonomic keys, in Atlantic waters and adjacent seas, the preadult and adult stages of *C. curtus* and *C. elongatus* can be distinguished from *L. salmonis* by the presence of lunules (Figure 1). Adult *C. curtus* can be distinguished from *C. elongatus* by differences in the shape of the genital complex and abdomen (Figure 1), differences in the setae on the distal margin of the exopod of the first leg (Figure 2), as well as differences in the number of setae on the exopod of the fourth leg (Figure 3). The fourth leg has four setae in *C. curtus* and five setae in *C. elongatus*. Species identification can be confirmed by reference to Parker et al. (1968), Kabata (1979, 1988) and Piasecki (1996). Earlier developmental stages of *C. elongatus* can be identified by reference to Piasecki (1996). The earlier developmental stages of *C. curtus* have not been described.

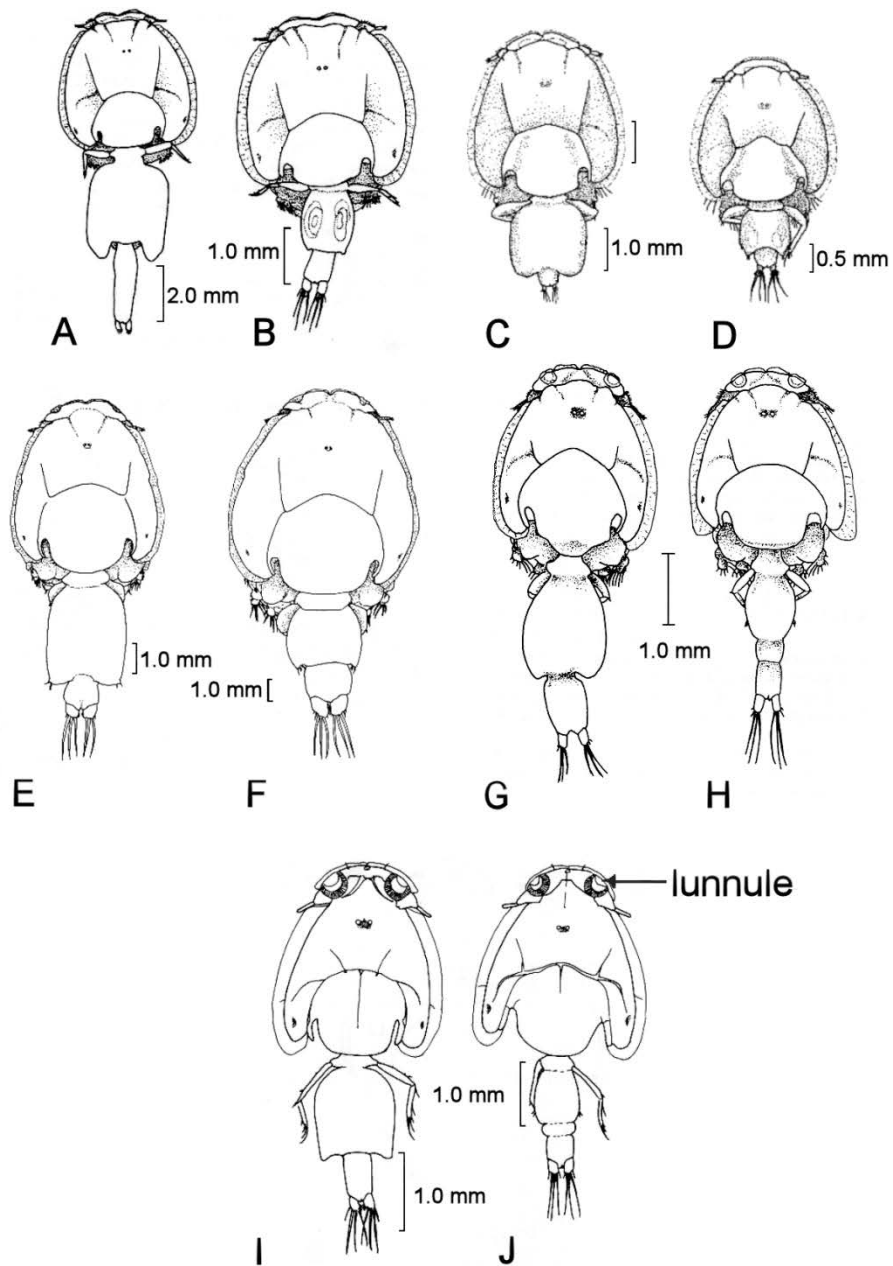


Figure 1. Adult stages of sea lice reported from wild and pen-reared salmon and trout in North America: (A) *Lepeophtheirus salmonis*, female; (B) same, male; (C) *Lepeophtheirus cuneifer*, female, (D) same, male, (E) *Caligus curtus*, female; (F) same, male; (G) *Caligus elongatus*, female; (H) same, male. (I) *Caligus clemensi*, female; (J) same, male. (A, B, E, F, G, H, I and J adapted from Kabata (1988); C and D adapted from Johnson and Albright (1991b)).

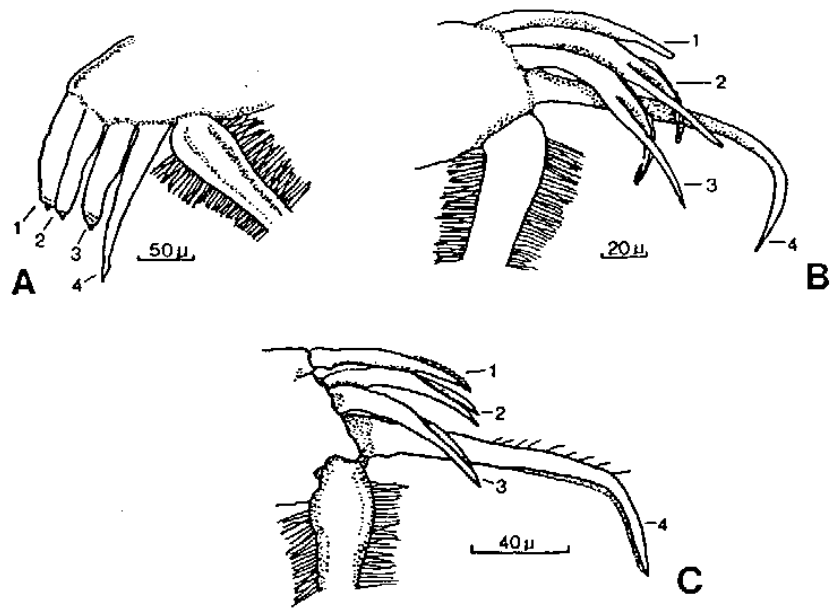


Figure 2. Distal margin of the exopod of the first leg: (A) *Caligus curtus*; (B) *Caligus clemensi*; (C) *Caligus elongatus* (A, C, modified from Kabata 1979).

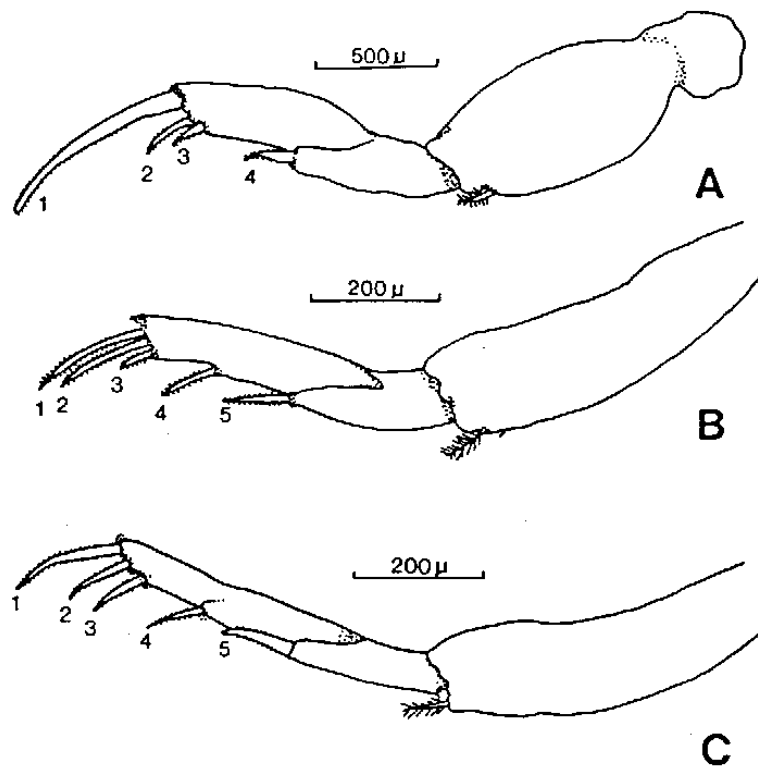


Figure 3. Structure of the fourth leg: (A) *Caligus curtus*; (B) *Caligus clemensi*; (C) *Caligus elongatus* (A, redrawn from Kabata 1979; B, redrawn from Kabata 1972; C, original).



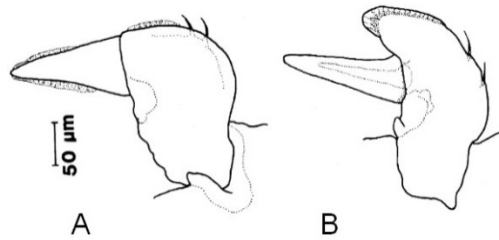


Figure 4. Basal spine of exopod of third leg of *Lepeophtheirus salmonis* (A) and *Lepeophtheirus cuneifer* (B) (adapted from Johnson and Albright 1991b).

Similarly on the Pacific coast, the preadult and adult stages of *C. clemensi* can be distinguished from those of *Lepeophtheirus* spp. by the presence of lunules (Figure 1). Preadult and adult stages of *L. salmonis* can be distinguished from *L. cuneifer* by the position of the large spine on the exopod of the third leg (Figure 4). There are two ways to identify *C. clemensi* chalimus stages from those of *Lepeophtheirus* spp. All of the chalimus stages can be identified to genus by examination of the anterior margin of the cephalothorax and the structure of the frontal filament. In *Lepeophtheirus* spp. the frontal filament is replaced at each of the molts and it appears to be continuous with the anterior margin of the cephalothorax. In *Caligus* spp. additional material is added to the frontal filament at each of the molts resulting in a series of segment-like sections where the filament meets the cephalothorax. In *Caligus* spp. the filament does not appear as continuous with the anterior margin of the cephalothorax. In the chalimus III and IV stages of *C. clemensi* rudimentary lunules are present. A detailed key for identifying the early developmental stages of *L. salmonis* from *C. clemensi* is given in Galbraith (2004).

The most significant limitation of the taxonomic keys designed to identify species of sea lice is that they permit only the identification of adult (male and female) stages. Although, copepodid and chalimus of sea lice belonging to *Lepeophtheirus* spp. are readily distinguished from those of *Caligus* spp. The lack of an accurate way to identify copepodid and chalimus stages of closely related species is problematic, as many of the sea lice occurring on wild-caught juvenile salmon are at these stages of development.

As mentioned above there is no description of the copepodid and chalimus stages of *L. cuneifer* a species that can be found along with *L. salmonis* on salmonid and non-salmonid hosts (Jones and Prospero-Porta, 2011; Jones et al., 2006). The use of ribosomal or mitochondrial gene sequences provide a bar-coding tool that permits the identification of these earlier development stages of eight distinct species of *Lepeophtheirus* and *Caligus* occurring on salmonid and non-salmonid species off western Canada (Jones et al. 2006, Jones and Prospero-Porta 2011). The primer sequences and the PCR cycling conditions that can be used to distinguish between all developmental stages of sea lice species found on salmonids in British Columbia are given in Jones and Prospero-Porta (2011).

Although, *L. salmonis* is the dominant sea lice species found on salmonids on the East Coast of Canada infections with *C. elongatus* and *C. curtus* can occur. As mentioned above the developmental stages of *C. elongatus* have been described but there are no descriptions of the early developmental stages of *C. curtus*. A molecular method to distinguish between species of *Caligus* has been developed and is reported in Øines and Heuch (2005).

### Identification of Juvenile and Adult Salmon

It is now widely acknowledged that juvenile salmon, regardless of species, are at greater risk due to sea lice infestations shortly after migrating to the ocean. The skill required to accurately

identify salmon to species at the post-smolt stage is gained from knowledge of morphological and meristic characteristics, habitat preferences and from experience, the latter being particularly relevant for the identification of local variants. The freshwater parr stage of most Pacific salmon possess distinctive patterns of colouration and markings (Edgell et al., 1997), however, these features tend to become less distinctive following migration of smolts into the ocean sea water. Parr markings, however, remain a reliable guide for juvenile pink and chum salmon for up to four weeks after migrating to the ocean. A more comprehensive taxonomic key for the identification of juvenile salmonids is found in (McConnell and Snyder, 1972).

Sub-adult and adult Pacific and Atlantic salmon are identified and can be distinguished from each other and other salmonids by using the taxonomic keys provided in Scott and Crossman (1973). Although identification is often practical by using a simplified identification scheme such as shown in Figure 5, the occurrence of local colour, meristic and morphological variants can make identification challenging for people with limited experience.

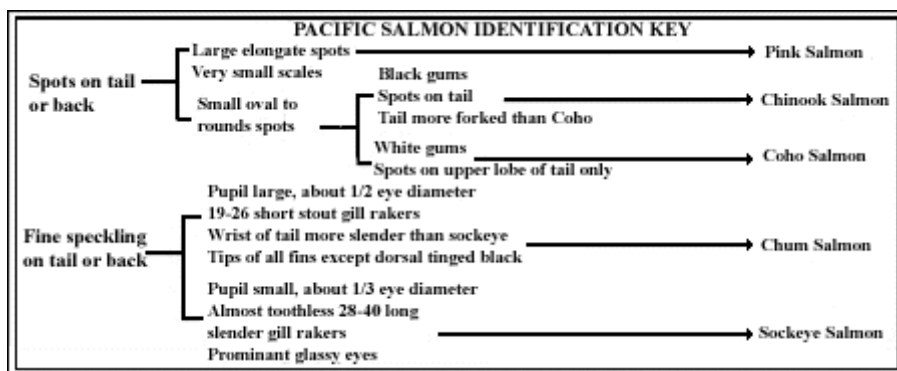


Figure 5. Simplified key for the identification of sub-adult and adult Pacific salmon. Source: [British Columbia Research Corporation](#).

## PROTOCOLS FOR THE MANAGEMENT, DISSEMINATION AND ANALYSIS OF DATA RESULTING FROM MONITORING PROGRAMS

There is good evidence that open sharing of data and research publications contributes to science and society in general by: enabling more rapid advances in scientific knowledge, by making stakeholders and other members of the public better informed, and ultimately enabling better decision making. In the areas of ecology and fisheries management, including sea lice research, there is in particular the need for long term data sets that are well documented, accessible so that new data can be added and re-analysis of data sets can occur and that are maintained over time in a secure location. Unfortunately, many data sets have become lost once projects have been completed, when data storage systems are replaced or when researchers retire or switch fields of interest.

Numerous sampling programs that have examined sea lice levels on wild salmonids have been summarized (Saksida et al., 2015; Table 2). Historically there has not been a strong interest in sharing of scientific information on sea lice outside of publications. It is important to remember that this is a situation that is not unique to sea lice research but is common across many disciplines. In fact in many respects sea lice data are already more accessible than other fisheries/ ecological data. For the last several years aquaculture companies in British Columbia have been posting summaries of their sea lice data on their company websites:

- [Mainstream Canada reports](#)
- [Marine Harvest reports](#)

---

In addition public reporting of sea lice numbers is also available from [DFO Aquaculture Management Division](#). With respect to the Broughton Archipelago summaries of the research results of the [DFO-lead Pink Salmon Action Plan](#) are available on the web for the years 2005 - 2009 with data for 2003 - 2004 available on request. Since 2010 monitoring activities for sea lice in the Broughton Archipelago have continued under the auspices of the [Broughton Archipelago Monitoring Program \(BAMP\)](#). This plan is being co-delivered and co-sponsored by the aquaculture industry, government, conservationists and academic researchers. Summaries of the results obtained under that program are available at the link above. As part of these activities a database, presently under development, will provide access to more detailed farm and field program sea lice data for the period of 2003 - onwards.

The development and maintenance of shared sea lice data resources requires the identification of an organization or group that is willing to take on the long-term responsibility and commitment to provide infrastructure, database management support and other funding. At this time no such group or organization has been identified. Associated with such a data set is the need for the development of clear data sharing policies that are agreeable to all parties who have contributed to the dataset. It would be expected that users of the data will acknowledge the source, as well as abide by any terms or conditions of use as set out in the data sharing policy. In addition, a method to effectively monitor adherence to the policy should be considered.

Table 2. Field sampling methods that have been used for capture of wild juvenile and adult Pacific and Atlantic salmon for sea lice enumeration. Modified from the original source: BC Salmon Forum P&G 2 field sampling methods for juvenile and adult Pacific salmon 21/02/06.

Gear Type	Sample Areas	Target Species Fish Size	Vessel and Personnel Requirements	Application and Benefits	Limitations
Dipnets	Near shore and offshore surface waters	Juvenile salmon	Small boat 1 person	Can sample individual fish Low cost	Very difficult to get a representative sample of the population. High risk of bias towards collection of poor performing fish
Beach seine	Surface waters in near shore areas <5 m deep	Juvenile salmon and other near shore fish species	Small boat 3 people	Can sample shallow water areas Can sample small fish Gear costs low	Limiting to use in near shore environments and therefore depending on the distribution of the population may not provide a representative sample of the population. Some loss or transfer of motile lice may occur. Difficult to estimate size of host population
Purse seine	Surface waters in near shore and offshore areas >5 m deep	Juvenile and adult salmon as well as other pelagic fish species	Medium to large commercial fishing vessel 3 - 4 people	Can sample surface waters >5M deep Can sample many species and age classes of fish dependent on net mesh size Can be used to obtain an relative abundance of a target species (e.g., Catch per unit effort (CPUE))	Cannot be used in waters that are <5 m deep. Limited to sampling surface waters the proportion of the population residing a greater depths will not be sampled. Difficult to fish in high current situations Some loss or transfer of motile lice may occur Medium to high cost due to vessel requirements
Trawling	Surface to deep waters	Juvenile and adult salmon as well as other pelagic and demersal fish species	Large vessels with sufficient horsepower and specialized gear for trawling 10+ people	Can sample all water column depths. Samples large volumes of water over relatively short periods of time. Can be used to estimate abundance of target species assuming capture efficiency of the trawl is known.	Cannot be used in waters <25 m deep. Due to net damage there is scale and sea lice loss which makes this gear type unacceptable for population studies. Cannot fish in confined or restricted areas Very high cost due to the requirement for a large vessel.
Trolling	Waters >5 m	Salmon > 25 cm	Medium sized vessel equipped with specialized gear 3 people	Can sample fish individually with minimal sea lice loss and no lice transfer Can fish in most locations where water depth are > 5 m Can be used to obtain an relative abundance of a target species (e.g., Catch per unit effort (CPUE))	Low sample size per day Moderate costs

Gear Type	Sample Areas	Target Species Fish Size	Vessel and Personnel Requirements	Application and Benefits	Limitations
Gill Net	Surface waters <5 m deep	Juvenile and adult salmon	Medium sized vessel equipped with specialized gear  3 people	Can sample surface waters <5M deep  Can be used in restricted areas  Can sample many species and age classes of fish dependent on net mesh size  Can be used to obtain an relative abundance of a target species (e.g., Catch per unit effort (CPUE))	Due to net damage there is often considerable scale and sea lice loss which makes this gear type unacceptable for population studies.
Ocean fish-lift trawl	Surface waters	Juvenile salmonids and other pelagic species	Medium sized vessel	Can sample surface waters  Reduced gear impact on salmon when compared to standard trawl  Suitable for sampling fish populations that are at risk	Some transfer of motile sea lice can occur  High cost for specialized gear and vessel.

Note: With all of these gear types it is likely that some loss of sea lice occurs during capture. However, the magnitude of such losses is not understood. Due to abrasion of fish during capture trawls and gill nets should not be used in programs to assess sea lice abundance.

Table 3. Summary of past and ongoing wild salmon sea lice monitoring programs in Canada.

Type of Study	Reference	Salmon Species Studied	Life History Stage	Sampling Method	Location	Sampling Dates (Frequency of Sampling)	Sea Lice Species Reported	Comments
<b>Atlantic Canada</b>								
BD	(Powell et al., 1999)	Atlantic	A	Trap	Penobscot River, Maine	May – October 1996 (I)	<i>Ls</i>	Fish were sampled in freshwater
BD, TA	(Lacroix and Knox, 2005)	Atlantic	PS	MTR	Bay of Fundy, Gulf of Maine	May – June 2001 – 2003 (S)	<i>Ce</i>	Ocean fish-lift type trawl used No <i>L. salmonis</i> found
<b>British Columbia</b>								
BD, DI	(Johnson et al., 1996)	Sockeye	A	G, PS	Alberni Inlet, Vancouver Island	Summers 1990 – 1992 (I)	<i>Ls</i>	Opportunistic sampling during test fisheries, skin damage assessed
BD	(Beamish et al., 2005a)	Coho Chinook Chum Sockeye Steelhead Pink	I	G, L	Central Bering Sea Central Pacific Ocean	June – July 2005 (S)	<i>Ls</i>	Gill net samples resulted in loss of some copepods
BD	(Beamish et al., 2005b)	Coho Chinook Chum Sockeye Pink	A	T	Queen Charlotte Strait	August 2004 August 2005 (S)	<i>Ls</i> <i>Cc</i>	
BD	(Beamish et al., 2005c)	Coho Chinook Chum Sockeye Pink	I/A	T	Johnstone Strait Queen Charlotte Strait Smith Inlet Rivers Inlet	August 2004 (S)	<i>Ls</i> <i>Cc</i>	Skin damage assessed
BD	(Gottesfeld et al., 2008)	Coho Chinook Chum Sockeye Pink	PS	MTR	Chatman Sound Skeena and Nass Estuaries	May – July 2007 (S)	<i>Ls</i> <i>Cc</i>	Ocean fish-lift trawl used

Type of Study	Reference	Salmon Species Studied	Life History Stage	Sampling Method	Location	Sampling Dates (Frequency of Sampling)	Sea Lice Species Reported	Comments
BD, Ht	(Butterworth et al., 2008)	Pink	PS	BS	Finlayson and Mathieson Channels	June 2004 (S)	<i>Ls</i> <i>Cc</i>	
BD, HT	(Morton et al., 2008)	Chum Pink Sockeye	PS	BS	Strait of Georgia Discovery Islands	April – June 2005-2006	<i>Ls</i> <i>Cc</i>	
BD, HT	(Morton et al., 2004)	Chum Pink	PS	DN, BS, PS	Bella Bella Prince Rupert Rivers Inlet Smith Inlet Broughton Archipelago	April – July 2002 (S, R)	<i>Ls</i> <i>Cc</i>	Different collection methods used at the different sites. Some sites in the Broughton Archipelago were sampled weekly for 10 weeks. Other sites were sampled only once.
BD, HT	(Morton et al., 2005)	Chum Pink	PS	DN	Broughton Archipelago	April – June 2002-2004 (R) extended into September in 2003 2002-2004 (R)	<i>Ls</i>	Appears that the at least part of the 2002 data set was the same as reported in Morton et al. (2004) as sites 4, 5 and 6
BD, HT	(Peet, 2007)	Chum Pink	PS	DN, BS	Finlayson and Mathieson Channels and adjacent inlets Broughton Archipelago Southern Gulf Islands	March – June 2003 - 2005	<i>Ls</i> <i>Cc</i>	Data for the Broughton Archipelago reported in Morton et al. (2004)
BD	(Trudel et al., 2007)	Coho Chinook Chum Sockeye Pink	PS/A	TR	Coastal waters of Oregon, Washington, British Columbia and Alaska	February- November 2002 and 2003	<i>Ls</i> <i>Cc</i>	1,339 surface tows, no repeat sampling, 30,305 fish examined only for preadult and adult stages, dominate species was <i>L. salmonis</i>
BD, HT	(Price et al., 2010)	Chum Pink	PS	BS	Finlayson and Mathieson Channels and adjacent inlets Southern Gulf Islands Discovery Islands	March – June 2007-2008 (R)	<i>Ls</i> <i>Cc</i>	

Type of Study	Reference	Salmon Species Studied	Life History Stage	Sampling Method	Location	Sampling Dates (Frequency of Sampling)	Sea Lice Species Reported	Comments
	(Price et al., 2011)	Sockeye	PS	MTR,BS	North Coast, Skeena Discovery Islands	May-July 2007 North Coast May-July 2008 Discovery Islands (R)	<i>Ls</i> <i>Cc</i>	Ocean fish-lift trawl used on North Coast
TA	(Gottesfeld et al., 2009)	Chinook Coho Pink	PS/A	D, MTR,T	Chatman Sound Skeena and Nass River Estuary	April – Aug 2004 - 2006 (smolts) (S) May-July 2006 (adults) (S)	<i>Ls</i> <i>Cc</i>	Different sampling methods used depending on life history stage and sample location Ocean fish-lift trawl used
TA, HT	(Saksida et al., 2011)	Chum Pink Others <sup>A</sup>	PS	BS	Kitasoo/ Xai'xais traditional territory Finlayson and Mathieson Channels	April – July 2005-2008 (R)	<i>Ls</i> <i>Cc</i>	Compared sea lice levels on wild and farmed salmonids
TA, HT	(Jones and Hargreaves, 2007)	Chum Pink	PS	BS, MPS	Broughton Archipelago	May – July 2004 – 2005 (R)	<i>Ls</i> <i>Cc</i>	Different sampling methods used depending on life history stage and sample location Sampling in this region continued under the Broughton Archipelago Monitoring Program
BD, HT	(Butterworth et al., 2008)	Pink	PS	BS	Finlayson and Mathieson Channels	June 2004 (S)	<i>Ls</i> <i>Cc</i>	
BD, TA	(Saksida et al., 2012)	Pink	PS	BS, MPS	Broughton Archipelago	April – June 2007 March – June 2008	<i>Ls</i> <i>Cc</i>	Samples from DFO-lead sampling program Full health assessment of sockeye salmon
BD, TA	(Anonymous , 2009)	Coho Chinook Chum Sockeye	PS	BS	Clayoquot Sound	Mid-March – June 2004 – 2007 (R)	<i>Ls</i> <i>Cc</i>	Chum salmon dominant species sampled
BD	(Beamish et al., 2009)	Coho Chinook Chum Sockeye Pink	PS	MS, TR	Gulf Islands Strait of Georgia	June – July 2008 (S)	<i>Ls</i> <i>Cc</i>	<i>Caligus clemensi</i> dominate species



Type of Study	Reference	Salmon Species Studied	Life History Stage	Sampling Method	Location	Sampling Dates (Frequency of Sampling)	Sea Lice Species Reported	Comments
TA, HT	Unpublished BAMP	Chum Pink Coho	PS	BS	Broughton Archipelago	May – June 2006 – 2012 (R)	Ls Cc	Continuation of DFO-lead surveillance in the Broughton Archipelago
BD, TA, HT	Unpublished PARR Program	Coho Chinook Chum Sockeye Pink	PS	MPS	Strait of Georgia Johnstone Strait	May – August 2010; May – July 2011 – 2012 (R)	Ls Cc	3 year PARR-funded program Full health assessment of sockeye salmon
BD	Unpublished	Chum	PS	BS	Muchalat Inlet and adjacent waters	May – June ongoing (R)	Ls Cc	Grieg Seafood Monitoring Program

**Type of Study:** BD – baseline data, DI – disease investigation, TA- trend analysis, HT – hypothesis testing,

**Life History Stage:** PS – post-smolt (< 4 months at sea), I – immature, A – adult (returning adult)

**Method of Capture:** G – commercial gillnet, L – long line, PS – commercial purse seine, MPS – modified purse seine, BS – beach seine, D – dip net, T – commercial troll, TR – trawl, MTR – modified trawl, TRAP – fishway trap.

**Sea Lice Species:** Ls – *Lepeophtheirus salmonis*, Cc – *Caligus clemensi*, Ce – *Caligus elongatus*

**Frequency of Sampling:** I – infrequent/opportunistic, S – single site samples, R – repeat sampling at specified sites/zones

<sup>A</sup> coho and sockeye salmon not reported by host species

---

## REFERENCES

- Anonymous 2006. Protocols & Guidelines A Reference Manual for Research Involving Wild/Cultured Fish Interactions with Sea Lice. 2 – Field sampling methods for juvenile and adult Pacific salmon and caligid zooplankton. BC Pacific Salmon Forum. 53 p. Appendix 1.
- Anonymous 2009. [Prevalence and density of sea louse \(\*L. salmonis\* and \*C. clemensi\*\) infections in juvenile chum salmon \(\*Oncorhynchus keta\*\) in Clayoquot Sound, 2004-2007](#). 31 p.
- Aretxabaleta, A.L., McGillicuddy, D.J., Smith, K.W., Manning, J.P., and Lynch, D.R. 2009. Model simulations of the Bay of Fundy Gyre: 2. Hindcasts for 2005-2007 reveal interannual variability in retentiveness. *J. Geophys. Res. Oceans* 114, C09005.
- Aretxabaleta, A.L., Smith, K.W., McGillicuddy, D.J., and Ballabrera-Poy, J. 2011. Model initialization in a tidally energetic regime: A dynamically adjusted objective analysis. *Ocean. Model.* 36: 219-227.
- Beamish, R., Wade, J., Pennell, W., Gordon, E., Jones, S., Neville, C., Lange, K., Sweeting, R. 2009. A large, natural infection of sea lice on juvenile Pacific salmon in the Gulf Islands area of British Columbia, Canada. *Aquaculture* 297: 31-37.
- Beamish, R.J., Ambers, N., and Hunter, K.L. 2005a. Sea lice counts on Pacific salmon caught in the Central North Pacific Ocean and Bering Sea aboard the R/V Wakatake Maru using gillnets and long line gear in July 2005. (NPAFC Doc. 873). 12p. Pacific Biological Station, Fisheries and Oceans Canada, 3190 Hammond Bay Road, Nanaimo, BC, V9T 6N7.
- Beamish, R.J., Ambers, N., Hunter, K.L., and Neville, C. 2005b. A comparison of sea lice on individually caught adult Pacific salmon in the coastal waters of British Columbia in August of 2004 and 2005. (NPAFC Doc. 874). 15 p. Fisheries and Oceans Canada, Science Branch - Pacific Region, Pacific Biological Station, Nanaimo, B.C., Canada V9T 6N7.
- Beamish, R.J., Lange, K.L., Neville, C.M., Sweeting, R.M., and Beacham, T.D. 2011. Structural patterns in the distribution of ocean- and stream-type juvenile chinook salmon populations in the Strait of Georgia in 2010 during the critical early marine period. NPAFC Doc. 1354. 27 pp.
- Beamish, R.J., Neville, C.M., Sweeting, R.M., and Ambers, N. 2005c. Sea lice on adult Pacific salmon in the coastal waters of Central British Columbia, Canada. *Fish. Res.* 76: 198-208.
- Beamish, R.J., Neville, C.M., Sweeting, R.M., Jones, S.R.M., Ambers, N., Gordon, E.K., Hunter, K.L., and McDonald, T.E. 2007. A proposed life history strategy for the salmon louse, *Lepeophtheirus salmonis* in the subarctic Pacific. *Aquaculture* 264: 428-440.
- Beamish, R.J., Pearsall, I.A., and Healy, M.C. 2003. A history of the research on the early marine life of Pacific salmon off Canada's Pacific Coast. NPAFC Bull. 3: 1-40.
- Bredin, K.A., Gerriets, S.H., and Guelpen, L.V. 2004. Distribution of rare, endangered and keystone marine vertebrates species in Bay of Fundy seascapes. Environment Canada, *In Health of the Bay of Fundy: Assessing key issues. Proceedings of the 5<sup>th</sup> Bay of Fundy Science Workshop and Coastal Forum "Taking the Pulse of the Bay"*, Wolfville, Nova Scotia, May 13-16, 2002. Edited by P.G. Wells, G.R. Daborn, J.A. Harvey, and S.J. Rolston. Environment Canada - Atlantic Region, Occasional Report No. 21, Dartmouth, Nova Scotia and Sackville New Brunswick. pp. 83-98.

- 
- Brooks, K.M. 2005. The effects of water temperature, salinity, and currents on the survival and distribution of the infective copepodid stage of sea lice (*Lepeophtheirus salmonis*) originating on Atlantic salmon farms in the Broughton Archipelago of British Columbia, Canada. *Rev. Fish. Sci.* 13: 177-204.
- Brooks, K.M., and Stucchi, D.J. 2006. The effects of water temperature, salinity and currents on the survival and distribution of the infective copepodid stage of the salmon louse (*Lepeophtheirus salmonis*) originating on Atlantic salmon farms in the Broughton Archipelago of British Columbia, Canada (Brooks, 2005) - A response to the rebuttal of Krkosek *et al.* (2005a). *Rev. Fish. Sci.* 14: 13-23.
- Bush, A.O., Lafferty, K.D., Lotz, J.M., and Shostak, A.W. 1997. Parasitology meets ecology on its own terms: Margolis *et al.* revisited. *J. Parasitol.* 83: 575-583.
- Butterworth, K.G., Cubitt, K.F., and McKinley, R.S. 2008. The prevalence, density and impact of *Lepeophtheirus salmonis* (Kroyer) infestation on juvenile pink salmon (*Oncorhynchus gorbuscha*) from the central coast of British Columbia, Canada. *Fish. Res.* 91: 35-41.
- Chang, B.D., Page, F.H., Losier, R.J., Lawton, P., Singh, R., and Greenberg, D.A. 2007. Evaluation of bay management area scenarios for the southwestern New Brunswick salmon aquaculture industry. *Can. Tech. Rep. Fish. Aquat. Sci.* 2722: v + 69 p.
- Chittenden, C.M., Beamish, R.J., Neville, C.M., Sweeting, R.M., and McKinley, R.S. 2009. The Use of Acoustic Tags to Determine the Timing and Location of the Juvenile Coho Salmon Migration out of the Strait of Georgia, Canada. *Trans. Am. Fish. Soc.* 138: 1220-1225.
- Dadswell, M.J. 2004. [Migration of inner Bay of Fundy Atlantic salmon in relation to the proposed quarry in the Digby Neck Region of Nova Scotia.](#) Wolfville, Nova Scotia. 46 p.
- Dempson, J.B., Robertson, M.J., Pennell, C.J., Furey, G., Bloom, M., Shears, M., Ollerhead, L.M.N., Clarke, K.D., Hinks, R., and Robertson, G.J. 2011. Residency time, migration route and survival of Atlantic salmon *Salmo salar* smolts in a Canadian fjord. *J. Fish Biol.* 78: 1976-1992.
- DFO. 2011. Wild salmon populations in the vicinity of a proposed finfish aquaculture development in St. Mary's Bay, Nova Scotia. *DFO Can. Sci. Advis. Sec. Sci. Resp.* 2011/001.
- DFO. 2013. Guidelines on Defining Potential Exposure and Associated Biological Effects from Aquaculture Pest and Pathogen Treatments: Anti-Sea Lice Bath Treatments in the Bay of Fundy, New Brunswick. *DFO. Can. Sci. Advis. Sec. Sci. Advis. Rep.* 2012/070.
- Edgell, P., Groot, C., Harman, C.F., and Pollard, W.R. 1997. *Field Identification of Coastal Juvenile Salmonids.* Harbor Publishing, Mafeira Park.
- Foreman, M.G.G., Czajko, P., Stucchi, D.J., and Guo, M. 2009. A finite volume model simulation for the Broughton Archipelago, Canada. *Ocean Model.* 30: 29-47.
- Foreman, M.G.G., Stucchi, D.J., Garver, K.A., Tuele, D., Issac, J., Grime, T., Guo, M., and Morrison, J. 2012. A circulation model for the Discovery Islands, British Columbia. *Atmos. Ocean.* 50: 301-316.
- Foreman, M.G.G., Stucchi, D.J., Zhang, Y., and Baptista, A.M. 2006. Estuarine and tidal currents in the Broughton Archipelago. *Atmos. Ocean* 44: 47-63.
- Galbraith, M. 2004. Identification of larval stages of *Caligus clemensi* and *Lepeophtheirus salmonis* from the Broughton Archipelago. *Can. Tech. Rep. Fish. Aquat. Sci.* 2548: 21p.

- 
- Galbraith, M., Johnson, S.C., and Jones, S. 2006. Standards, Protocols & Guidelines for Research Involving Wild/Cultured Fish Interactions with Sea Lice. 1 – Sea Lice Biology, Identification and Laboratory Methods. British Columbia Pacific Salmon Forum. 16 p. Appendix 2.
- Glover, K.A., Hamre, L.A., Skaala, O., and Nilsen, F. 2004. A comparison of sea louse (*Lepeophtheirus salmonis*) infection levels in farmed and wild Atlantic salmon (*Salmo salar* L.) stocks. *Aquacult.* 232: 41-52.
- Gottesfeld, A.S., Carr-Harris, C., Proctor, B., and Rolston, D. 2008. Sockeye salmon juveniles in Chatham Sound, Report to the Pacific Salmon Forum July 2008. Sjeeba Fisheries Commission, Hazelton, B.C., Canada.
- Gottesfeld, A.S., Proctor, B., Rolston, L.D., and Carr-Harris, C. 2009. Sea lice, *Lepeophtheirus salmonis*, transfer between wild sympatric adult and juvenile salmon on the north coast of British Columbia, Canada. *J. Fish Dis.* 32: 45-57.
- Groot, C. 2011. Migration routes and survival rates of sockeye and pink salmon in the Strait of Georgia and Discovery Islands. Report for Contract PO #F1625-110310. Yellow Point Bio-Research, Gabriola Island.
- Groot, C. and Cooke, K. 1987. Are the migration of juvenile and adult Fraser River sockeye salmon (*Oncorhynchus nerka*) in near-shore waters related? In *Sockeye salmon (Oncorhynchus nerka) population biology and future management*. Edited by H.D. Smith, L. Margolis, and C.C. Wood. *Can. Spec. Pub. Fish. Aquat. Sci.* 96. pp. 53-60.
- Groot, C., Cooke, K., Ellis, G., and Bailey, R. 1985. Data record of juvenile sockeye salmon and other fish species captured by purse seine and trawl in the Strait of Georgia, Johnstone Strait and Queen Charlotte straits in 1982, 1983, 1984 and 1985. *Can. Data Rep. Fish. Aquat. Sci.* 561.
- Heuch, P.A., Revie, C.W., and Gettinby, G. 2003. A comparison of epidemiological patterns of salmon lice, *Lepeophtheirus salmonis*, infections on farmed Atlantic salmon, *Salmo salar* L., in Norway and Scotland. *J. Fish Dis.* 26: 539-551.
- Holst, J.C. and McDonald, A. 2000. FISH-LIFT: a device for sampling live fish with trawls. *Fish. Res.* 48: 87-91.
- Johnson, S.C. and Albright, L.J. 1991a. The developmental stages of *Lepeophtheirus salmonis* (Krøyer, 1837) (Copepoda: Caligidae). *Can. J. Zool.* 69: 929-950.
- Johnson, S.C. and Albright, L.J. 1991b. *Lepeophtheirus cuneifer* Kabata, 1974 (Copepoda: Caligidae) from seawater-reared rainbow trout, *Oncorhynchus mykiss*, and Atlantic salmon, *Salmo salar* in the Strait of Georgia, British Columbia, Canada. *Can. J. Zool.* 69: 1414-1416.
- Johnson, S.C., Blaylock, R.B., Elphick, J., and Hyatt, K.D. 1996. Disease induced by the sea louse (*Lepeophtheirus salmonis*) (Copepoda: Caligidae) in wild sockeye salmon (*Oncorhynchus nerka*) stocks of Alberni Inlet, British Columbia. *Can. J. Fish. Aquat. Sci.* 53: 2888-2897.
- Jones, S.R.M. and Beamish, R.J. 2012. Comment on "Evidence of farm-induced parasite infestations on wild juvenile salmon in multiple regions of coastal British Columbia, Canada". *Can. J. Fish. Aquat. Sci.* 69: 201-203.
- Jones, S.R.M. and Hargreaves, N.B. 2007. The abundance and distribution of *Lepeophtheirus salmonis* (Copepoda: caligidae) on pink (*Oncorhynchus gorbuscha*) and chum (*O. keta*) salmon in coastal British Columbia. *J. Parasitol.* 93: 1324-1331.
-

- 
- Jones, S. and Johnson, S. 2014. Sea lice monitoring and non-chemical measures A: Biology of sea lice, *Lepeophtheirus salmonis* and *Caligus* spp., in western and eastern Canada. DFO Can. Sci. Advis. Sec. Res. Doc. 2014/019. iv + 18 p.
- Jones, S.R.M. and Prosperi-Porta, G. 2011. The diversity of sea lice (Copepoda: Caligidae) parasitic on threespine stickleback (*Gasterosteus aculeatus*) in coastal British Columbia. *J. Parasitol.* 97: 399-405.
- Jones, S.R.M., Prosperi-Porta, G., Kim, E., Callow, P., and Hargreaves, N.B. 2006. The occurrence of *Lepeophtheirus salmonis* and *Caligus clemensi* (Copepoda: Caligidae) on three-spine stickleback *Gasterosteus aculeatus* in coastal British Columbia. *J. Parasitol.* 92: 473-480.
- Kabata, Z. 1972. Developmental stages of *Caligus clemensi* (Copepoda: Caligidae). *J. Fish. Res. Brd. Can.* 29: 1571-1593.
- Kabata, Z. 1973. Species of *Lepeophtheirus* (Copepoda: Calidigae) from Fishes of British Columbia. *J. Fish. Res. Brd. Can.* 30: 729-759.
- Kabata, Z. 1974. *Lepeophtheirus cuneifer* sp. nov. (Copepoda: Caligidae), a parasite of fishes from Pacific coast of North America. *J. Fish. Res. Brd. Can.* 31: 43-47.
- Kabata, Z. 1979. Parasitic copepoda of British fishes. Ray Society, London.
- Kabata, Z. 1988. Copepoda and Branchiura. *In* Guide to the parasites of fishes of Canada. Part II Crustacea. Edited by L. Margolis and Z. Kabata. *Can. Spec. Pub. Fish. Aquat. Sci.* 101: 3-127.
- Krkosek, M., Lewis, M.A., Morton, A., Frazer, L.N., and Volpe, J.P. 2006. Epizootics of wild fish induced by farm fish. *Proc. Natl. Acad. Sci. U.S.A.* 103: 15506-15510.
- Krkosek, M., Lewis, M.A., and Volpe, J.P. 2005a. Transmission dynamics of parasitic sea lice from farm to wild salmon. *Proc. Roy. Soc. B Sci.* 272: 689-696.
- Krkosek, M., Morton, A., and Volpe, J.P. 2005b. Nonlethal assessment of juvenile pink and chum salmon for parasitic sea lice infections and fish health. *Trans. Am. Fish. Soc.* 134: 711-716.
- Lacroix, G.L. 2008. Influence of origin on migration and survival of Atlantic salmon (*Salmo salar*) in the Bay of Fundy, Canada. *Can. J. Fish. Aquat. Sci.* 65: 2063-2079.
- Lacroix, G.L. and Knox, D. 2005. Distribution of Atlantic salmon (*Salmo salar*) postsmolts of different origins in the Bay of Fundy and Gulf of Maine and evaluation of factors affecting migration, growth, and survival. *Can. J. Fish. Aquat. Sci.* 62: 1363-1376.
- Lacroix, G.L., Knox, D., and Stokesbury, M.J.W. 2005. Survival and behaviour of post-smolt Atlantic salmon in coastal habitat with extreme tides. *J. Fish Biol.* 66: 485-498.
- Lefevre, M.A., Stokesbury, M.J.W., Whoriskey, F.G., and Dadswell, M.J. 2012. Atlantic salmon post-smolt migration routes in the Gulf of St. Lawrence. *ICES J. Mar. Sci.* 69: 981-990.
- McConnell, R.J. and Snyder, G.R. 1972. Key to field identification of anadromous juvenile salmonids in the Pacific Northwest. NOAA Tech. Rep. NMFS CIRC-366. 6 p.
- Melnychuk, M.C., Welch, D.W., and Walters, C.J. 2010. Spatio-temporal migration patterns of Pacific salmon smolts in rivers and coastal marine waters. *Plos One* 5: e12916.
- Morton, A., Routledge, R., and Krkosek, M. 2008. Sea louse infestation in wild juvenile salmon and Pacific herring associated with fish farms off the east-central coast of Vancouver Island, British Columbia. *North Am. J. Fish. Manage.* 28: 523-532.
-

- 
- Morton, A., Routledge, R., McConnell, A., and Krkosek, M. 2011. Sea lice dispersion and salmon survival in relation to salmon farm activity in the Broughton Archipelago. *ICES J. Mar. Sci.* 68: 144-156.
- Morton, A., Routledge, R., Peet, C., and Ladwig, A. 2004. Sea lice (*Lepeophtheirus salmonis*) infection rates on juvenile pink (*Oncorhynchus gorbuscha*) and chum (*Oncorhynchus keta*) salmon in the nearshore marine environment of British Columbia, Canada. *Can. J. Fish. Aquat. Sci.* 61: 147-157.
- Morton, A., Routledge, R.D., and Williams, R. 2005. Temporal patterns of sea louse infestation on wild Pacific salmon in relation to the fallowing of Atlantic salmon farms. *North Am. J. Fish. Manage.* 25: 811-821.
- Morton, A.B. and Williams, R. 2003. First report of a sea louse, *Lepeophtheirus salmonis*, infestation on juvenile Pink Salmon, *Oncorhynchus gorbuscha*, in nearshore habitat. *Can. Field Nat.* 117: 634-641.
- Øines, O. and Heuch, P.A. 2005. Identification of sea louse species of the genus *Caligus* using mtDNA. *J. Mar. Biol. Assoc. U.K.* 85: 73-79.
- Brewer-Dalton, K. (ed), Page, F.H., Chandler, P., and Ratsimandresy, A. 2014. Oceanographic conditions of salmon farming areas with attention to those factors that may influence the biology and ecology of sea lice, *Lepeophtheirus salmonis* and *Caligus* spp., and their control. DFO Can. Sci. Advis. Sec. Res. Doc. 2014/048. vi + 48 p.
- Page, F.H., Chang, B.D., Losier, R.J., Greenberg, D.A., Chaffey, J.D., and McCurdy, E.P. 2005. Water circulation and management of infectious salmon anemia in the salmon aquaculture industry of southern Grand Manan Island, Bay of Fundy. *Can. Tech. Rep. Fish. Aquat. Sci.* 2595: iii + 78 p.
- Parker, R.R., Kabata, Z., Margolis, L., and Dean, M.D. 1968. A review and description of *Caligus curtus* Müller, 1785 (Caligidae: Copepoda) type species of its genus. *J. Fish. Res. Brd. Can.* 25: 1923-1969.
- Parker, R.R. and Margolis, L. 1964. A new species of parasitic copepod, *Caligus clemensi* sp. nov. (Caligoida, Caligidae), from pelagic fishes in the coastal water of British Columbia. *J. Fish. Res. Brd. Can.* 21: 873-889.
- Peet, C.R. 2007. Interactions between sea lice (*Lepeophtheirus salmonis* and *Caligus clemensii* sic), juvenile salmon (*Oncorhynchus keta* and *Oncorhynchus gorbuscha*) and salmon farms in British Columbia., Thesis (M.Sc.), Department of Biology. University of Victoria, Victoria.
- Piasecki, W. 1996. The developmental stages of *Caligus elongatus* von Nordmann, 1832 (Copepoda: Caligidae). *Can. J. Zool.* 74: 1459-1478.
- Powell, K., Trial, J.G., Dube, N., and Opitz, M. 1999. External parasite infestation of sea-run Atlantic salmon (*Salmo salar*) during spawning migration in the Penobscot River, Maine. *Northeastern Natural.* 6: 363-370.
- Preikshot, D., Beamish, R.J., Sweeting, R., Neville, C.M., and Beacham, T.D. 2012. The residence time of juvenile Fraser River sockeye salmon in the Strait of Georgia. *Mar. Coast. Fish. Dyn. Manage. Eco. Sci.* 4: 438-449.
- Price, M.H.H., Morton, A., and Reynolds, J.D. 2010. Evidence of farm-induced parasite infestations on wild juvenile salmon in multiple regions of coastal British Columbia, Canada. *Can. J. Fish. Aquat. Sci.* 67: 1925-1932.
-

- 
- Price, M.H.H., Proboszcz, S.L., Routledge, R.D., Gottesfeld, A.S., Orr, C., and Reynolds, J.D. 2011. Sea louse infection of juvenile sockeye salmon in relation to marine salmon farms on Canada's West Coast. *Plos One* 6: e16851.
- Price, M.H.H. and Reynolds, J.D. 2012. Salmon farms as a source of sea lice on juvenile wild salmon; reply to the comment by Jones and Beamish. *Can. J. Fish. Aquat. Sci.* 69: 204-207.
- Ratsimandresy, A.W., Page, F., Mabrouk, G., Losier, R., Drover, D., Ings, D., and McCurdy, P. 2012. Aquaculture Drifter Programme: Progress Update 2010. DFO Can. Sci. Advis. Sec. Res. Doc. 2011/127. vi + 41 p.
- Ritchie, G. 1997. The host transfer ability of *Lepeophtheirus salmonis* (Copepoda: Caligidae) from farmed Atlantic salmon, *Salmo salar* L. *J. Fish Dis.* 20: 153-157.
- Saksida, S., Bricknell, I., Robinson, S. and Jones, S. 2015. Population ecology and epidemiology of sea lice in Canadian waters. DFO Can. Sci. Advis. Sec. Res. Doc. 2015/004. v + 34 p.
- Saksida, S.M., Greba, L., Morrison, D., and Revie, C.W. 2011. Sea lice on wild juvenile Pacific salmon and farmed Atlantic salmon in the northernmost salmon farming region of British Columbia. *Aquaculture* 320: 193-198.
- Saksida, S.M., Marty, G.D., Jones, S.R.M., Manchester, H.A., Diamond, C.L., Bidulka, J., and St-Hilaire, S. 2012. Parasites and hepatic lesions among pink salmon, *Oncorhynchus gorbuscha* (Walbaum), during early seawater residence. *J. Fish Dis.* 35:137-151.
- Schram, T.A. 1993. Supplemental descriptions of the developmental stages of *Lepeophtheirus salmonis* (Krøyer, 1837) (Copepoda: Caligidae). In *Pathogens of wild and farmed fish: sea lice*. Edited by G.A. Boxshall and D. Defaye. Ellis Horwood. Chichester, pp. 30-50.
- Schram, T.A. 2004. Practical identification of pelagic sea lice larvae. *J. Mar. Biol. Assoc. U.K.* 84: 103-110.
- Scott, W.B. and Crossman, E.J. 1973. *Freshwater Fishes of Canada*. Bull. Fish. Res. Brd. Can. 184. 966 p.
- Sheehan, T.F., Renkawitz, M.D., and Brown, R.W. 2011. Surface trawl survey for U.S. origin Atlantic salmon *Salmo salar*. *J. Fish Biol.* 79: 374-398.
- Stucchi, D.J., Guo, M., Foreman, M.G.G., Czajko, P., Gallbraith, M., Mackas, D., and Gillibrand, P.A. 2011. Modeling sea lice production and concentration in the Broughton Archipelago, British Columbia. In: *Salmon Lice: An integrated Approach to Understanding Parasite Abundance and Distribution*. Edited by S. Jones and R. Beamish. John Wiley & Sons, Chichester, pp. 117-150.
- Trudel, M., Jones, S.R.M., Thiess, M.E., Morris, J.F.T., Welch, D.W., Sweeting, R.M., Moss, J.H., Wing, B.L., Farley, E.V.J., Murphy, J.M., Baldwin, R.E., and Jacobson, K.C. 2007. Infestations of motile salmon lice on Pacific salmon along the West Coast of North America. *Amer. Fish. Soc. Symp.* 57: 157-182.
- Tucker, C.S., Sommerville, C., and Wootten, R. 2002. Does size really matter? Effects of fish surface area on the settlement and initial survival of *Lepeophtheirus salmonis*, an ectoparasite of Atlantic salmon *Salmo salar*. *Dis. Aquat. Org.* 49: 145-152.

---

# **APPENDIX 1: STANDARDS, PROTOCOLS & GUIDELINES FOR RESEARCH INVOLVING WILD/CULTURED FISH INTERACTIONS WITH SEA LICE**

## **1 – Sea Lice Biology, Identification and Laboratory Methods**

*By: Moira Galbraith, Dr. Stewart Johnson and Dr. Simon Jones*

---

- A. INTRODUCTION
    - A.1 Developmental Stages and Lifecycle
    - A.2 Sea Lice Biology
    - A.3 Feeding
    - A.4 Growth and Survival
    - A.5 Reproduction, Egg Production and Hatching
  - B. SEA LICE IDENTIFICATION
    - B.1 Species Keys
  - C. LABORATORY METHODS
    - C.1 Laboratory processing and enumeration of sea lice
    - C.2 Fixation of copepods
    - C.3 Fixation for long-term storage
    - C.4 Molecular Taxonomy of Sea Lice
    - C.5 Data reporting
      - C.5.1 Definitions
      - C.5.2 Examination of Fish and Enumeration of Sea lice
      - C.5.3 Quantification of Damage Caused by Sea Lice
  - D. LABORATORY RING TEST
  - E. REFERENCES
-



---

## Acknowledgements

The authors wish to thank the following who have contributed to this document: Luis Afonso (National Research Council, Canada), Kevin Butterworth (University of British Columbia, Centre for Aquaculture and the Environment, AquaNet), Alan Donald (Statistician), Mark Fast (National Research Council, Canada)

## A. INTRODUCTION

This SPG provides a brief overview of aspects of sea lice biology and simplified methods to identify the difference species found on salmonids in British Columbia. The term sea louse (pl. sea lice) is the common name used for several species of marine ectoparasitic copepods of the family Caligidae (Order Copepoda: Suborder Siphonostomatoida) that infect fish. In B.C. these species include *Caligus clemensi*, *Lepeophtheirus salmonis*, and *Lepeophtheirus cuneifer* that have been reported from salmonids and non-salmonid hosts. In addition to these species there are another nine species of *Lepeophtheirus* and one species of *Caligus* reported from numerous non-salmonid hosts (Margolis and Arthur 1979, Kabata 1988; Johnson and Albright 1991a; McDonald and Margolis 1995). Emphasis here is only on *C. clemensi*, *L. salmonis*, and *L. cuneifer* that use wild and farmed salmonids in British Columbia waters as hosts.

For more detailed reviews of their sea lice biology readers should consult Pike and Wadsworth (1999), Tully and Nolan (2002), Johnson and Fast (2004) and Johnson et al. (2004). An identification key for the adults of species of sea lice found in British Columbia is given in Kabata (1988).

### A.1 Developmental Stages and Lifecycle

All sea lice species share a similar pattern of development (Figure 1). Eggs hatch into the first of two non-feeding naupliar stages. These stages are followed by a single copepodid stage that is non-feeding until it encounters a suitable host. The copepodid stage moults into the first of four chalimus stages, which are attached to the host by means of a frontal filament. Failure to attach to a host and begin feeding of the host will result in death due to starvation. Time to death is dependent on the water

temperature and activity level of the copepodid. After the chalimus stages there are one (*Caligus* species) or two (*Lepeophtheirus* species) pre-adult stages. These stages are similar in overall body form to the adults with males being easily differentiated from females at this stage. Pre-adults are free-moving on the host with exception of short periods at the moult when temporary frontal filaments are produced. In all species there is a single free-moving adult stage, which does not undergo any further moulting.

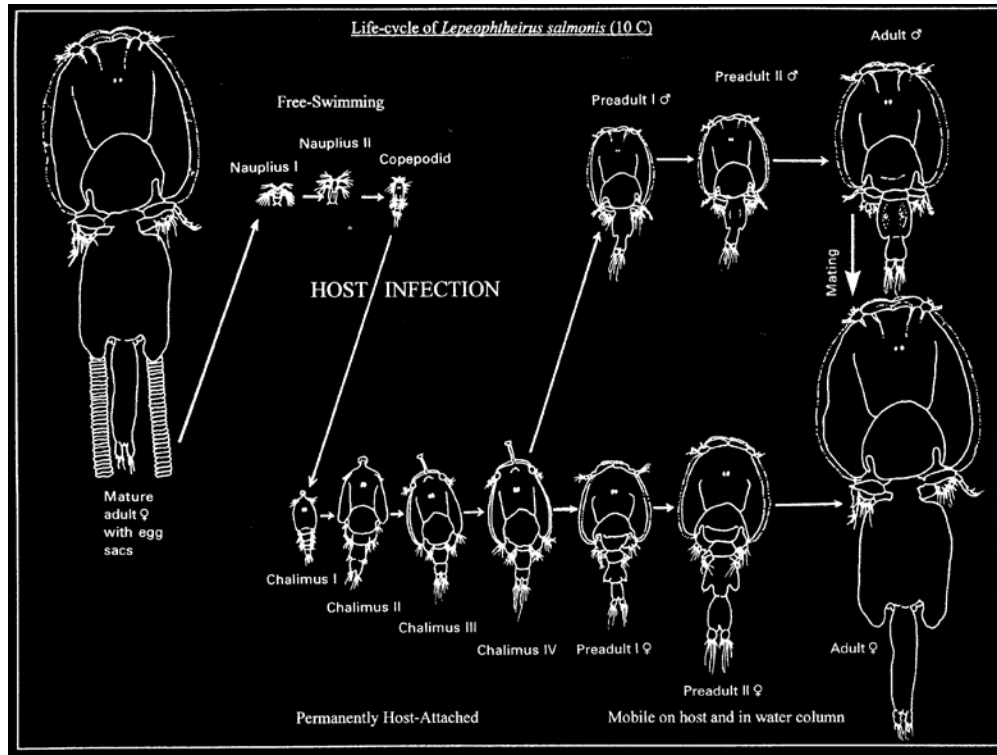


Figure 1. Life cycle of *Lepeophtheirus salmonis* from (A. Shinn and J. Bron unpublished).

In all species the primary infectious stage is the copepodid, however all species are known to transfer between hosts as preadults and adults (Bruno and Stone 1990). Such transfers appear to be more common in *C. clemensi* and *L. cuneifer*.

Detailed descriptions of the lifecycles and developmental stages of *C. clemensi* and *L. salmonis* are given in Parker and Margolis (1964), Kabata (1972) and Johnson and Albright (1991b). Supplemental descriptions of *L. salmonis* based on specimens collected from European waters are given in Schram (1993, 2004). Although there is a good description of the adult stages of *Lepeophtheirus cuneifer* (see Kabata 1974) the earlier developmental stages of this species are yet to be described.

## A.2 Sea Lice Biology

The biology of *L. salmonis* has been thoroughly reviewed in a number of articles (Pike and Wadsworth 1999, Tully and Nolan 2002, Johnson and Fast 2004, Johnson et al. 2004). With exception of their host ranges there is little known about the biology of *C. clemensi* and *L. cuneifer*. Several important aspects of sea lice biology are reviewed briefly here.

## A.3 Feeding

Once attached to the host sea lice feed on host mucus, skin and blood using rasping mouthparts contained within the oral cone. Heavy infections can successively remove mucus and skin to expose underlying muscle, fin rays and/or bone. In heavy infections morbidity and death can occur due to osmoregulatory failure, blood loss, and/or the development of secondary diseases (Johnson and Fast 2004). The relationship of the number of sea lice to severity of the disease is dependent on:

- 1) the species, size and age of the fish,

- 2) the general state of health of the fish, and
- 3) the species and developmental stages of sea lice that are present and their relative abundance.

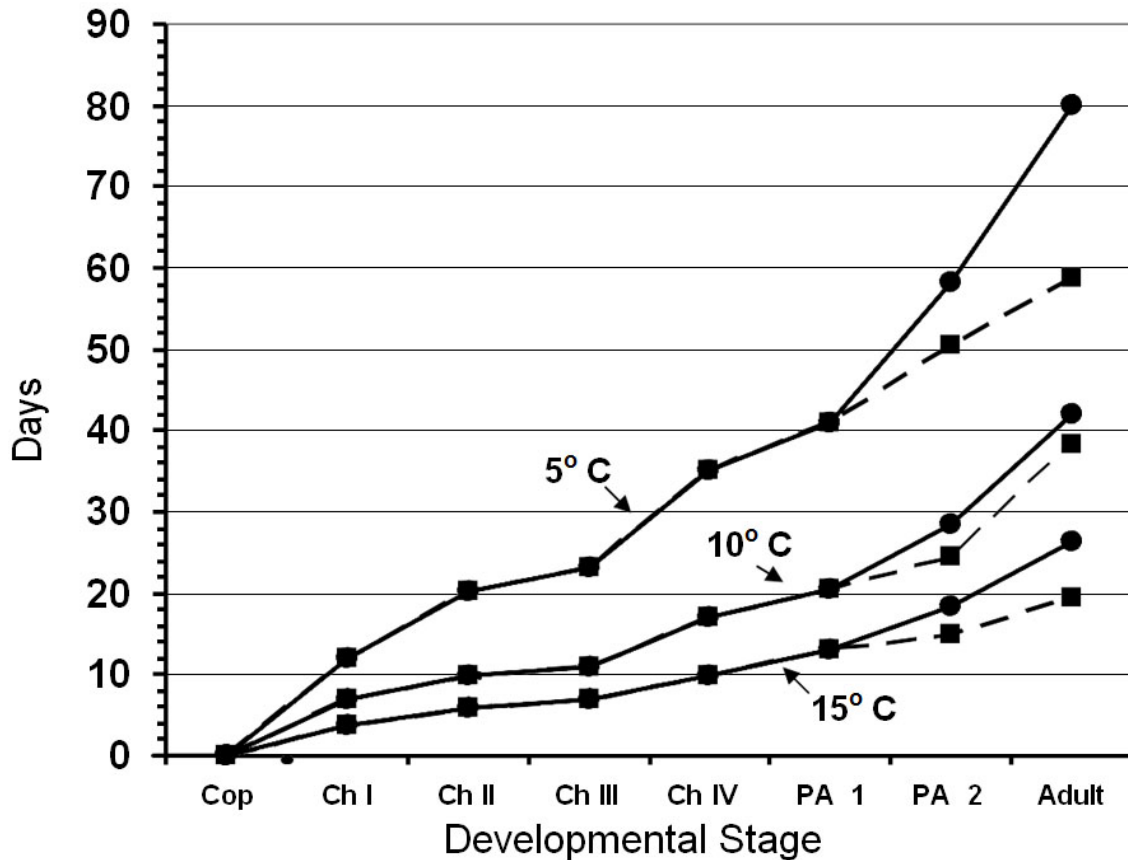


Figure 2. Approximate development times of *Lepeophtheirus salmonis* from the copepodid to adult stage on Atlantic salmon. Note at all temperatures males develop faster than females. Solid lines are females, dashed lines are males. Cop – copepodid, Ch I – first chalimus, Ch II – second chalimus, Ch III – third chalimus, Ch IV – fourth chalimus, PA 1.

#### A.4 Growth and Survival

The development rate of embryos and the non-feeding naupliar and copepodid stages of sea lice is primarily controlled by water temperature, although other factors such as salinity may have some effect. The development rate of the attached stages (copepodid through adults), in addition to being affected by temperature and other environmental variables, is also affected by host factors which vary among host species. For example, Johnson (1993) reported that *L. salmonis* developed faster on Atlantic salmon than Chinook salmon. *Lepeophtheirus salmonis* development times from egg to adult male and female have been determined (Figure 2) (Johnson and Albright 1991b; Johnson 1993). Development times for *C. clemensi* and *L. cuneifer* are unknown.

#### A.5 Reproduction, Egg Production and Hatching

The mating of *L. salmonis* involves the transfer of a spermatophore from the adult male to the adult female. Once mated females can produce as many as six batches of egg strings without

---

further mating over a 50-day period at 14°C. Sea lice carry their eggs as long strings that trail from the posterior edge of the genital segment. The number of *L. salmonis* eggs reported per string is remarkably variable, depending on the species of sea lice, as well as environmental (seasonal) and host factors. *Caligus clemensi* and *L. cuneifer* produce fewer eggs per female (<200) than *L. salmonis* females, which can produce from 100 to over 1200 eggs. A major factor controlling the number of eggs produced is temperature, whereas other environmental factors such as photoperiod can influence both egg numbers as well as egg size (Pike and Wadsworth, 1999; Heuch et al., 2000). Species of host, host maturation state physiological factors can also affect the number of eggs. The viability of nauplii developing from an individual female reportedly differs according to the generation number of the egg sac and the environment under which the eggs are incubated. For example incubation of egg strings in water with a salinity of < 15 parts per thousand (ppt) results in the failure of egg strings to produce viable nauplii. For additional details on reproduction, egg production and hatching readers should refer to Pike and Wadsworth (1999), Heuch et al. (2000), Tully and Nolan (2002) and Johnson and Fast (2004) and references therein.

## **B. SEA LICE IDENTIFICATION**

Features such as size, colour, position on host, and species of host that they are found on cannot be used to differentiate species of sea lice. Identification must be based on morphological features such as overall body shape and appendage structure. These features vary between species as well as between the different developmental stages.

### **B.1 Species Keys**

A simplified key to aid in the identification of preadult and adult stages of *Caligus clemensi*, *Lepeophtheirus salmonis* and *Lepeophtheirus cuneifer* is provided below.

1. Lunules present (Figure 3)..... *Caligus clemensi*  
    Lunules absent ..... 2
  
2. Large spine of the basal segment of the exopod of the third leg inserts at the distal tip of the segments outgrowth (Figure 4)..... *Lepeophtheirus salmonis*  
    Large spine of the basal segment of the exopod of the third leg inserts at the midpoint of the segment's outgrowth (Figure 4)..... *Lepeophtheirus cuneifer*

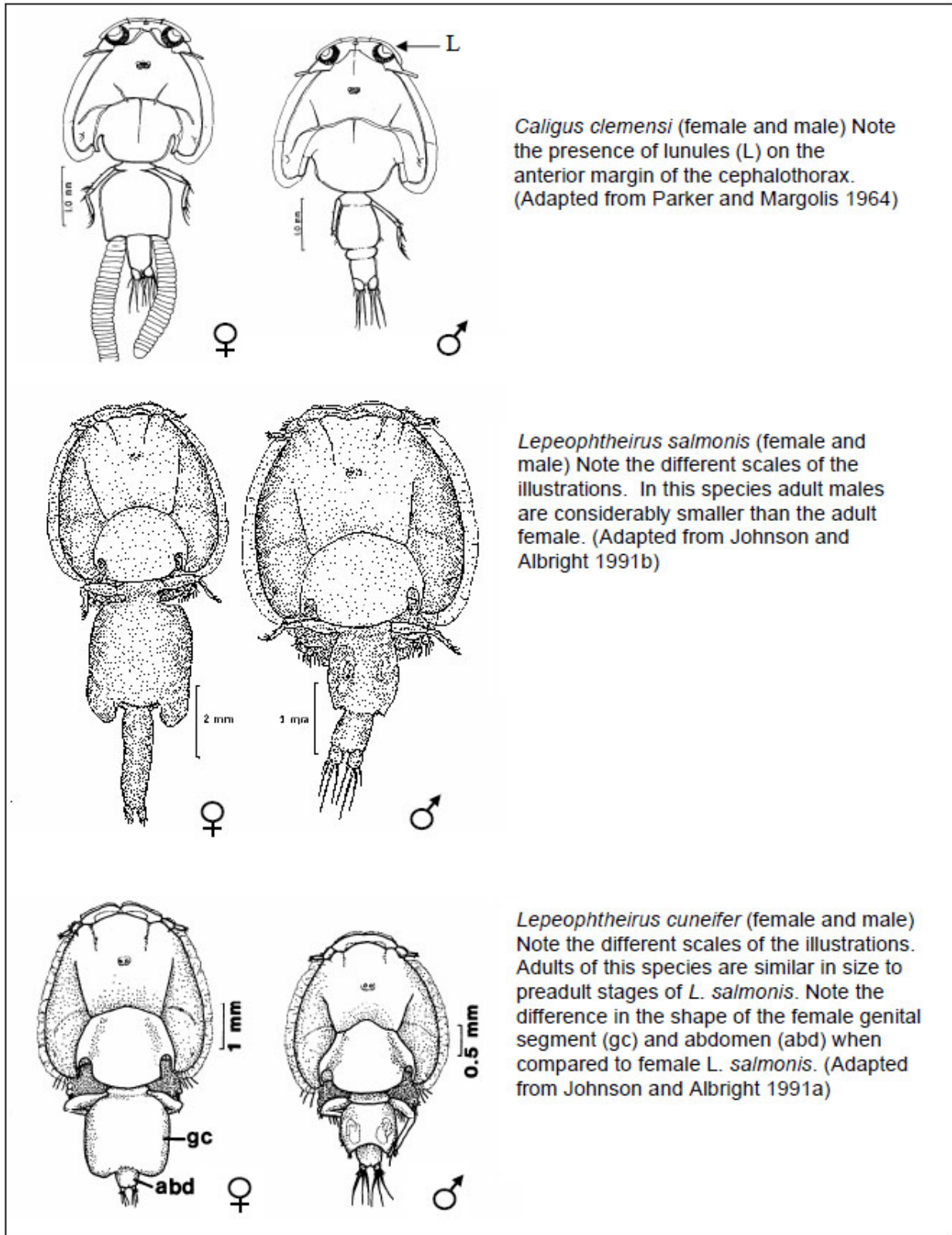


Figure 3. Adult stages of sea lice found on salmonids in British Columbia waters.

Lunules absent ..... 2.0

2.0 Large spine of the basal segment of the exopod of the third leg inserts at the distal tip of the segments outgrowth (Figure 4) ..... *Lepeophtheirus salmonis*

Large spine of the basal segment of the exopod of the third leg inserts at the midpoint of the segment's outgrowth (Figure 4). ..... *Lepeophtheirus cuneifer*

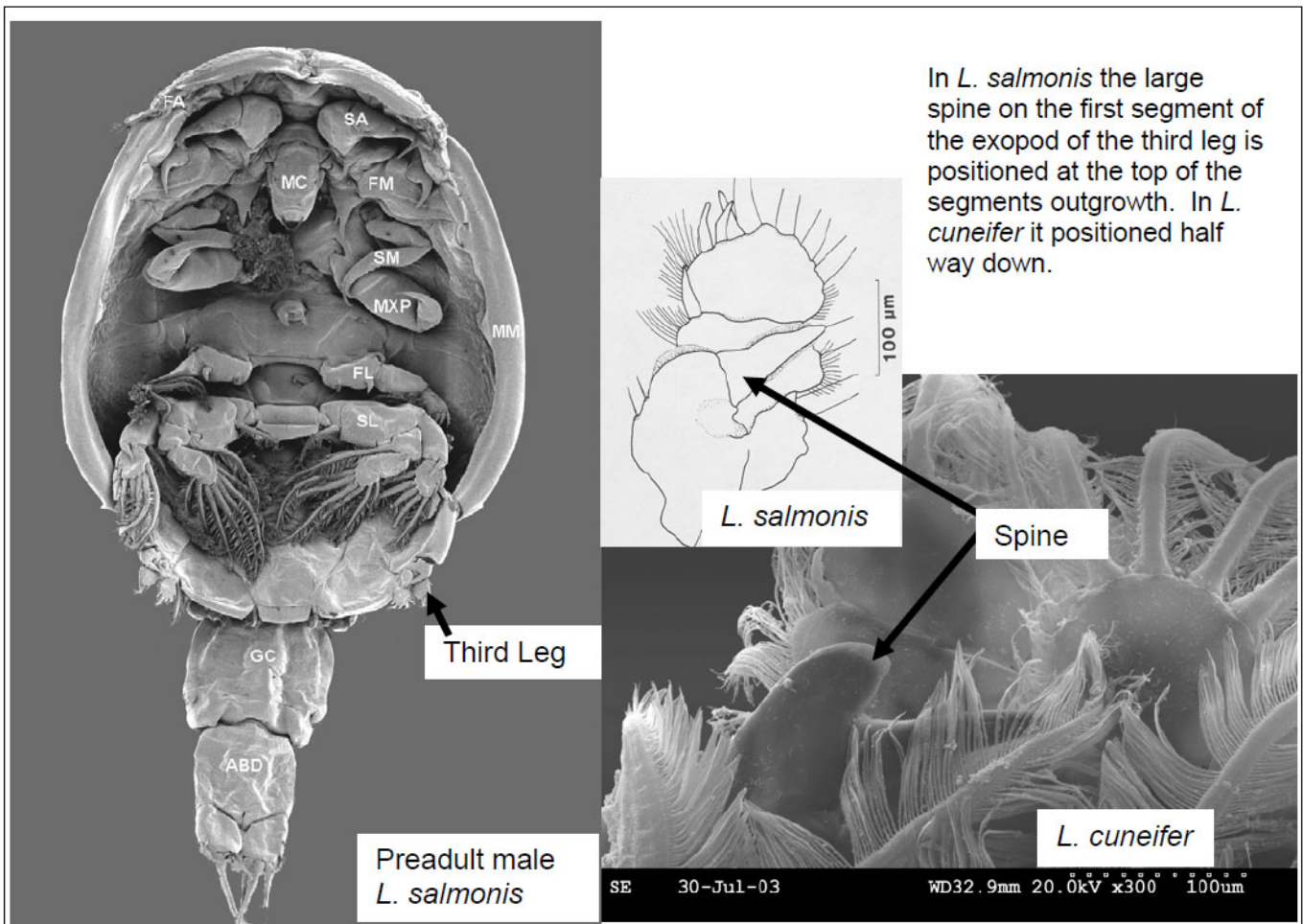


Figure 4. Preadult and adult *Lepeophtheirus salmonis* can be distinguished from preadult and adult *Lepeophtheirus cuneifer* by the position of the large spine on the basal segment of the exopod of the third leg.

---

Complete morphological descriptions of the preadult and adult stages are given for:

- *Caligus clemensi* in: Parker and Margolis, 1964; Kabata 1972; Galbraith 2005
- *Lepeophtheirus salmonis* in: Johnson and Albright, 1991b; Schram, 1993; Galbraith 2005
- *Lepeophtheirus cuneifer* in: Kabata, 1974 (adults only).

Identification of the earlier developmental stages is more difficult. The earlier (nauplii to chalimus IV) developmental stages of *L. salmonis* were described in Johnson and Albright 1991b and Schram 1993. *Caligus clemensi* nauplii to chalimus IV stages are described in Kabata (1972).) There are no descriptions of the earlier developmental stages of *L. cuneifer*.

There are two ways to identify *C. clemensi* chalimus stages from those of *Lepeophtheirus* species. All of the chalimus stages can be identified to genus by examination of the anterior margin of the cephalothoraxes and the structure of the frontal filament. In *Lepeophtheirus* spp. the frontal filament is replaced at each of the moults and it appears to be continuous with the anterior margin of the cephalothoraxes (Figure 5).

In *Caligus* sp. additional material is added to the frontal filament at each of the moults. This results in a series of segment-like sections where the filament meets the cephalothorax. The filament does not appear as continuous with the anterior margin of the cephalothorax. In the chalimus III and IV stages of *C. clemensi* rudimentary lunules are present (Figure 5). A detailed key for identifying the early developmental stages of *L. salmonis* from *C. clemensi* is given in Galbraith (2005).

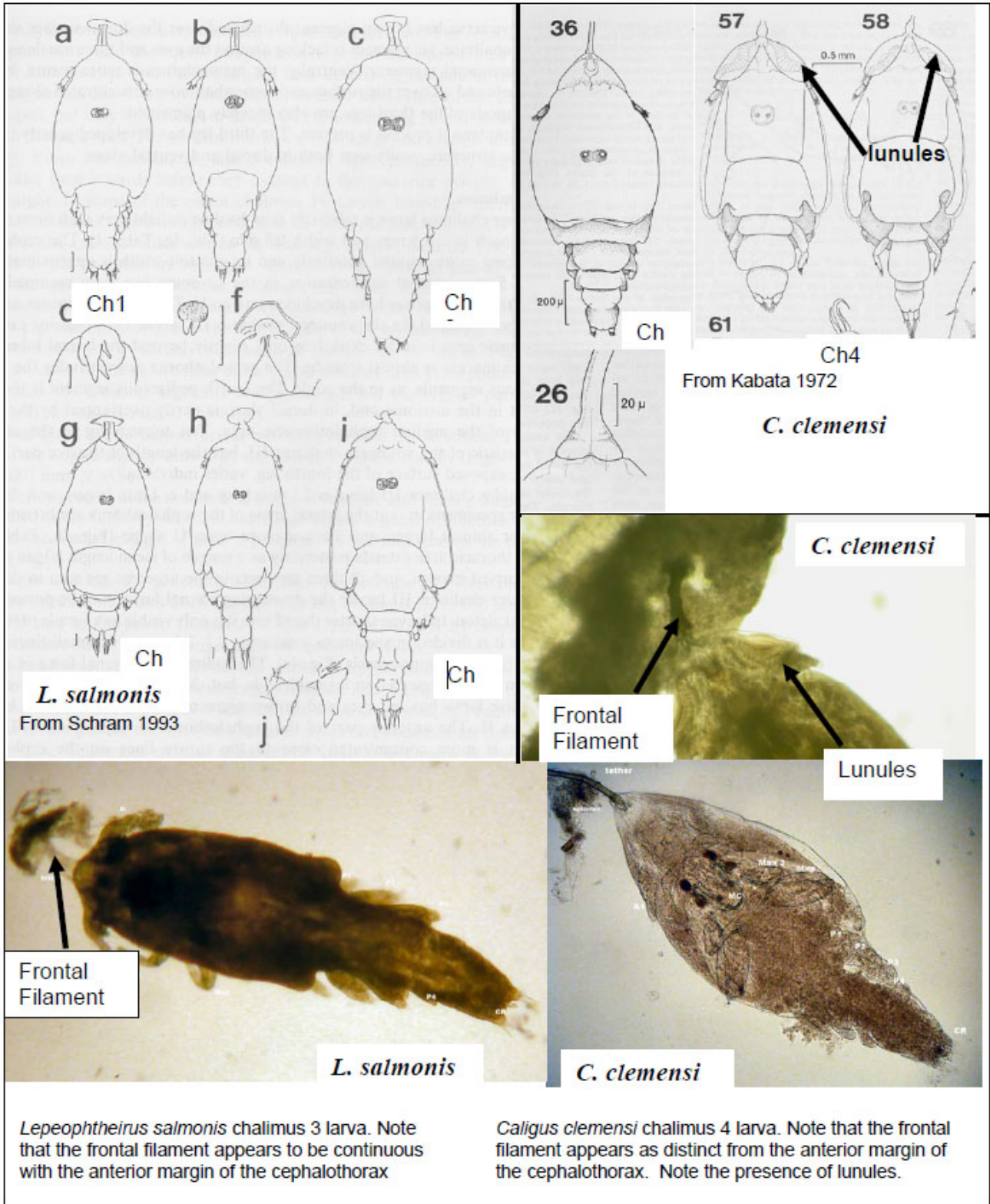


Figure 5. Chalimus stages of *Lepeophtheirus salmonis* and *C. clemensi*, including differences in the structure of the frontal filament.



---

## C. LABORATORY METHODS

### C.1 Laboratory processing and enumeration of sea lice

It is preferable to examine fish for the presence of sea lice while they are fresh and the lice still alive. However, in the field this is often not practical and fish and their associated sea lice are either fixed whole (in 10% neutral buffered formalin or >70% ethanol) or frozen. Fish can be killed by a lethal dose of anaesthetic followed by a blow to the head. Tricaine methanesulfonate (MS222) at a concentration of at least 0.1 g/L water is lethal. Once fish are completely immobile, remove them from the anaesthetic and administer a blow to the head. Any equipment that has been used to sample the fish and the anaesthetic bath must be carefully examined for sea lice that may have become dislodged. Although freezing of samples is quick and easy, subsequent enumeration, identification and staging of sea lice from frozen samples is more difficult as there may be deterioration of the samples during the process of freezing and defrosting. It is important to freeze fish individually in well sealed bags. This permits small numbers of fish to be thawed at a time and ensures that lice counts and species identification are made on lice from an individual host. Fish samples should not be held for prolonged periods at room temperature. Sea lice obtained from frozen samples once thawed need to be fixed rapidly in one of the fixatives outlined below. Samples of fish and sea lice that have been frozen are usable for taxonomic but not histological study.

Samples of fish that have been fixed in formalin or 70% ethanol should be removed from the fixative and transferred to water prior to enumeration of sea lice. The remaining fixative must be examined for sea lice that have become dislodged from the host.

Very small fish can be examined without dissection. For larger fish, the fins, opercula and gills may be dissected and examined separately prior to examining the remainder of the fish. As early developmental stages of sea lice are very small it is necessary to examine all of the body parts of the fish under a dissecting microscope or with a hand held lens to obtain accurate counts. Examination of the body segments and appendages of the early developmental stages for species identification requires a compound microscope with 250X magnification. The stage of development is assessed using a dissecting microscope with 50X magnification.

### C.2 Fixation of copepods

Copepods can be fixed in either a buffered solution of formalin (5 to 10%) or ethanol. A common buffering agent is Borax (sodium tetraborate -  $\text{Na}_2\text{B}_4\text{O}_7$ ). To make buffered formalin add 2 g of borax to 100 ml of formalin (40% formaldehyde solution) and mix until dissolved. The formalin is then diluted to the appropriate concentration. To keep samples that have been fixed in formalin for prolonged periods, they should be transferred within 10 days to an alcohol-based storage solution as described below.

Ethanol (EtOH) is a good fixative at concentrations between 70 and 95%. Samples for molecular analysis are normally fixed and stored in 95% ethanol.

### C.3 Fixation for long-term storage

Ideally, sea lice initially fixed in buffered formalin should be transferred within 10 days into 70% ethanol before long term storage. The addition of 1% glycerine to the 70% ethanol produces a better long term storage medium as it keeps the copepods flexible and provides some protection against drying out of the samples. Samples fixed initially in ethanol can remain in ethanol or be transferred to the 70% ethanol 1% glycerine solution. Methanol or isopropanol (rubbing alcohol) can be used in place of ethanol in the storage solution.

---

## C.4 Molecular Taxonomy of Sea Lice

Sea lice occurring on wild-caught juvenile Pacific salmon are most frequently copepodid and chalimus stages. Available knowledge allows these stages to be identified as *Lepeophtheirus* spp. or *Caligus* spp. The morphological criteria for distinguishing naupliar, copepodid and chalimus stages of the many species that occur in coastal B.C. are not available. Several laboratories (e.g. Marine Laboratory, Aberdeen; National Veterinary Institute, Oslo; Pacific Biological Station, Nanaimo) are exploring mitochondrial and genomic DNA sequences as taxonomic tools for sea lice identification. These methods may be useful to identify morphologically indistinguishable copepodid and chalimus stages (Øines and Heuch, 2005; Tjensvoll et al., 2005). Useable DNA samples can be obtained from sea lice that are alive, fresh frozen or fixed in 95% ethanol, as described above.

## C.5 Data reporting

### C.5.1 Definitions

**Host Terms:** A variety of terms are used to describe host length. These include: **Fork Length:** Measured from the tip of the jaw or tip of the snout with closed mouth to the center of the fork in the tail (caudal fin); **Total Length:** Measured from the most forward point of the head, with the mouth closed, to the farthest tip of the caudal fin; **Orbital Length:** Measured from the hind margin of the socket of the eye to the end of the caudal peduncle.

Fork length is the most common length reported in sea lice studies. Orbital length may be useful when reporting sea lice numbers from spawning salmonids as it overcomes problems associated with modification of snout region (especially in males) at spawning and loss of caudal fin tissue.

### Parasitological Terms

A variety of terms are commonly used when describing parasite numbers and their distribution on individual hosts and within host populations. Further information on these terms and other definitions are given in Bush et al. (1997). The following are the most widely accepted definitions of the most commonly used terms. The definitions quoted from Bush et al. (1997) are as follows:

1. **Prevalence** is “the number of hosts infected with 1 or more of a particular parasite species (or taxonomic group) divided by the number of hosts examined for that species”. Prevalence is commonly expressed as a percentage when used descriptively or as a proportion when used in mathematical models.

For example, 10 salmon are sampled and 5 of them have 1 (or more) *L. salmonis* on them. This gives a prevalence of *L. salmonis* of 50%.

2. **Density** is “the number of individuals of a particular parasite species in a measured sampling unit taken from a host or a habitat”. It is recommended that density be used when an accurate census of all parasites is difficult or impossible to make. When the host is used as a sampling unit the terms intensity and abundance should be used.

For example, 10 cubic meters of water are sampled for *L. salmonis* copepodids and 40 are found. The density of *L. salmonis* copepodids would be 4 copepodids per cubic meter.

3. **Intensity** (of infection) is “the number of individuals of a particular parasite species in or on a single infected host”. **Mean intensity** is “the average intensity of a particular species of parasite among the infected members of a particular host species. In other words, it is the

---

total number of parasites of a particular parasite species found in a sample divided by the number of **hosts infected** with that parasite”.

For example, examination of a single salmon reveals the presence of 10 *L. salmonis*. The **intensity** of infection is 10. If 15 salmon were examined and 10 of the fish were infected with a total of 50 *L. salmonis* then **mean intensity** would be 5 *L. salmonis* ( $50 \div 10$ ) Note: intensity can never be equal to 0.

4. **Abundance** is the “number of individuals of a particular parasite in/on a single host regardless of whether or not the host is infected”. **Mean abundance** is “the average abundance of a parasite species among all members of a particular host population”. In other words it “is the total number of individuals of a particular parasite species in a sample of a particular host species divided by the total number of hosts of that species examined”. For clarity it is recommended also that mode be reported with the mean abundance (Alan Donald, per. comm.). **Mode of abundance** is the abundance of a parasite species which appears most frequently among all members of a particular host population. The mode is significant in describing the infection of a population as often most individuals in a population may be infected at low levels, but only a couple of individuals may have very high infection levels, which would drive up the mean abundance value. Reporting the mode therefore provides a more representative description of the parasite numbers in the population.

For example, examination of a single salmon reveals the presence of 10 *L. salmonis*. The **abundance** of infection is 10. If there were no *L. salmonis* on the salmon then the **abundance** would be 0. If 15 salmon were examined and 10 of the fish were infected with a total of 50 *L. salmonis* then **mean abundance** would be 3.3 ( $50 \div 15$ ). If 13 salmon were examined and 10 fish had 1 *L. salmonis* each, 2 fish had 2 *L. salmonis* each, and 1 fish had 50 *L. salmonis*, the **mean abundance** would be 4.9 ( $64 \div 13$ ) and the **mode of abundance** would be 1.

#### C.5.2 Examination of Fish and Enumeration of Sea lice

The fork length and wet weight should be determined for each fish. In laboratory experiments and some field studies a variety of methods are used to overcome problems that arise when comparing sea lice infection levels on fish of different sizes. These range from correcting the number of copepods to a standard body length or weight to calculation of body surface area and reporting sea lice numbers per unit of area “lice infection density” (see Heuch et al. 2003). Body surface area can be determined using both image analysis and a model for body surface area: Body Surface Area ( $\text{cm}^2$ ) =  $a (W)^b$ , where  $a=12.05$ ,  $W$ = fish weight in grams, and  $b=0.61$  (e.g. Tucker et al. 2002; Glover et al. 2004). This equation has been used for both Atlantic salmon and brown trout. Other authors have used the same equation with slightly different values for  $a$  and  $b$ . The actual value used does not really matter unless comparisons between different data sets are desired.

Copepod numbers on the gills, fins and other body surfaces should be recorded separately as the physiological and/or immunological effects of the parasite vary with body region (e.g. chalimus larvae on the tips of fins have a lower impact than those living on the body or gills). As a minimum copepods need to be identified to species and identified to general stage (copepodid, chalimus, preadult and adult). Identification between the different developmental stages of chalimus larvae and preadults can be difficult. If necessary sub samples of sea lice for staging can be taken and fixed for later identification using the procedures outlined above (Section C.2).

---

Damage of the host including damage caused by sea lice should be noted (e.g. missing fins). Signs of other diseases should also be noted. Sea lice damage can be quantified as outlined below.

### **C.5.3 Quantification of Damage Caused by Sea Lice**

The severity of physical damage caused by the attachment and feeding activities of sea lice is related to:

- 1) the species of sea lice,
- 2) the number and developmental stages of the copepods,
- 3) their site of attachment,
- 4) the species of host and
- 5) host physiological status (e.g. stress level).

In general, damage by copepodid and chalimus larvae is limited to a small area around their point of attachment where they erode the epidermis and sub-epidermis (see Bron et al. 1991; Johnson and Albright 1992). However, heavy infections of chalimus stages of *C. clemensi* on wild pink salmon (*Oncorhynchus gorbuscha*) and *L. salmonis* on wild sea trout (*Salmo trutta*) have been associated with serious fin damage including complete fin removal (see Parker and Margolis 1964; Tully et al. 1993). Preadult and adult sea lice that are larger and capable of moving on the surface of the fish, cause more severe and widespread damage. Infected salmon may have evidence of lesions on their heads, beside and immediately behind the dorsal fin and in the perianal region (see Johnson et al. 1996). In seriously diseased salmonids open lesions in which the epidermis is breached and the underlying tissues exposed, commonly occur on the head and/or behind the dorsal fin (Jónsdóttir et al. 1992; Johnson et al. 1996).

A number of systems have been used to quantify sea lice damage on salmonids. These have been applied to the description of gross lesions. Quantification of gross damage is most accurate on fish that have not been frozen or preserved. Histological rankings have previously been developed to grade the severity of other fish diseases (e.g. Jones and Groman, 2001). However this approach has not been developed for sea lice infections. As with all histological assessments, it is critical that tissues are collected from fish immediately after death and that they are properly fixed and processed. Tissues that have been frozen or stored on ice for long periods of time cannot be used for histology. Methods for fixation of fish tissues for histology are given in SPG4.

Whichever ranking system is used the criteria for differentiating the ranks must be well documented and include examples of the different ranks. A single system of ranking for all users would be preferred for obvious reasons. If such a system can be agreed upon then photo examples could be distributed to various labs, and/or training sessions on lesion classification held.

The following ranking systems for gross body lesions have been included here as examples. A ranking system to classify gross sea lice lesions on different body regions of freshly caught and unfixed sockeye salmon has been developed (Figure 6; Johnson et al., 1996). Their ranking system was as follows:

---

**Position: Posterior to dorsal fin, base of dorsal fin, and head.**

<b>Rank</b>	<b>Description</b>
0	Unmarked
1	Grazing evident; discolouration of skin surface and in some cases mild descaling and hemorrhaging
2	Early white lesion; Partial removal of epidermis and scales, resulting in patches of grayish to whitish necrotic tissue.
3	White lesion; Epidermis and scales removed, lesions covered with continuous whitish necrotic tissue, no breaks in skin exposing musculature.
4	Early open lesion; White lesion with small (1-2 mm) point lesions open to musculature
5	Open lesion; Epidermis and dermis removed exposing musculature

**Position: Perianal region**

<b>Rank</b>	<b>Description</b>
0	Unmarked
1	Grazing evident; Characterized by the discolouration of skin surface, and in some cases blood seepage from the scale pockets
2	Mild descaling and hemorrhaging
3	Extensive discolouration, descaling and hemorrhaging
4	Early Open: Extensive descaling and hemorrhaging with small point lesions (1-2 mm) open to the musculature
5	Open lesion; Epidermis and dermis removed exposing musculature

A simpler ranking system has been used to describe gross lesions on Pacific salmon (Beamish et al., unpublished).

<b>Rank</b>	<b>Description</b>
0	No skin damage and no red discoloration of skin surface from hemorrhaging
1	Minor red discoloration from hemorrhaging, but reduced in intensity and area; no scale abrasion but pin hole penetrations may be present
2	Moderate hemorrhaging resulting in more red color over an area about one half the size of the anal fin, minor scale abrasion may be present
3	Severe hemorrhaging, area of hemorrhaging approximately the size of the anal fin or larger and almost uniformly red; no lesions; scale abrasion common, but skin intact
4	Lesions present, skin removed and muscle exposed or skin partially removed exposing necrotic tissue; hemorrhaging at margins of lesions

Differences in the distribution of ranks can be used to compare the levels of damage between different samples of fish as well as for other purposes. Ranking or lesion scores are typically not normally distributed; therefore data analysis will require either transformation or use of nonparametric statistics (e.g. Kruskal Wallis test) to determine whether differences are significant.

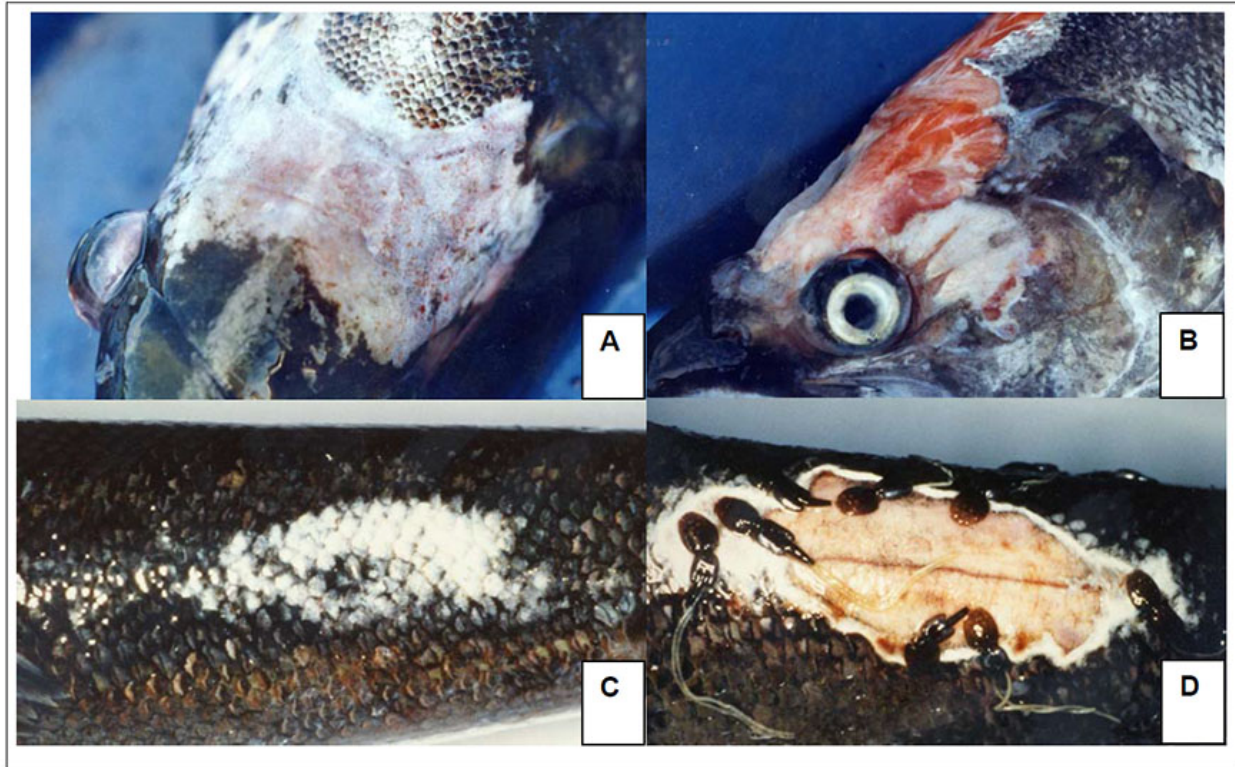


Figure 6. Classification of lesions caused by *Lepeophtheirus salmonis* on sockeye salmon (*Oncorhynchus nerka*). **A, Rank 3** - White lesion; Epidermis and scales removed, lesions covered with continuous whitish necrotic tissue, no breaks in skin exposing musculature. **B, Rank 5** - Open lesion; Epidermis and dermis removed exposing musculature; **C, Rank 2** - Early white lesion; Partial removal of epidermis and scales, resulting in patches of greyish to whitish necrotic tissue; **D, Rank 5** - Open lesion; Epidermis and dermis removed exposing musculature.

#### D. LABORATORY RING TEST

There is a need for a quality assurance system when difficult or subjective observations are being made (e.g. staging of chalimus larvae, extent of skin damage) especially in the field.

To verify or assess accuracy of laboratory taxonomic identification, a laboratory intercalibration, or ring test, could be established. This would involve sending a small sample of pre-identified (but not disclosed) copepods of each species to different experts/groups for verification and confirmation of life stages.

A similar method could also be used to see how researchers are rating lesions by sending a small sub sample of damaged fish (i.e. by lice) or detailed photographs to various labs for rating.

For field studies independent cross checking by multiple observers on the same samples should be undertaken on a random/unannounced basis.

#### E. REFERENCES

Bron, J. E., Sommerville, C., Jones, M. and Rae, G. H. 1991. The settlement and attachment of early stages of the salmon louse, *Lepeophtheirus salmonis* (Copepoda: Caligidae) on the salmon host, *Salmo salar*. J. Zool. 224: 201-212.

- 
- Bruno, D. W., and Stone, J. 1990. The role of saithe, *Pollachius virens* L., as a host for the sea louse, *Lepeophtheirus salmonis* Krøyer and *Caligus elongatus* Nordmann. *Aquaculture* 89: 201-207.
- Bush, A. O., Lafferty, K. D., Lotz, J. M., and Shostak A. W. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. *J. Parasitol.* 83(4) 575-583.
- Galbraith, M. 2005. Identification of larval stages of *Caligus clemensi* and *Lepeophtheirus salmonis* from the Broughton Archipelago. *Can. Tech. Rep. Fish. Aquat. Sci.* 2548: 20 pp.
- Glover, K. A., Hamre, L. A., Skaala, Ø. and Nilsen, F. 2004. A comparison of sea louse (*Lepeophtheirus salmonis*) infection levels in farmed and wild Atlantic salmon (*Salmo salar* L.) stocks. *Aquacult.* 232: 41- 52.
- Heuch, P. A., Nordhagen, J. R. and Schram, T. S. 2000. Egg production in the salmon louse [*Lepeophtheirus salmonis* (Kroyer)] in relation to origin and temperature. *Aquacult. Res.* 31: 805-814.
- Heuch, P.A., Revie, C.W. and Gettinby, G. 2003. A. comparison of epidemiological patterns of salmon lice, *Lepeophtheirus salmonis*, infections on farmed Atlantic salmon, *Salmo salar* L., in Norway and Scotland. *J. Fish Dis.* 26: 539-551.
- Johnson, S. C. and Albright, L. J. 1991a. *Lepeophtheirus cuneifer* Kabata, 1974 (Copepoda: Caligidae) from seawater-reared rainbow trout, *Oncorhynchus mykiss*, and Atlantic salmon, *Salmo salar*, in the Strait of Georgia, British Columbia, Canada. *Can. J. Zool.* 69: 1414-1416.
- Johnson, S. C. and Albright, L. J. 1991b. The developmental stages of *Lepeophtheirus salmonis* (Krøyer, 1837) (Copepoda: Caligidae). *Can. J. Zool.* 69: 929-950.
- Johnson, S. C. and Albright, L. J. 1991c. Development, growth, and survival of *Lepeophtheirus salmonis* (Copepoda: Caligidae) under laboratory conditions. *J. mar. biol. Ass. U.K.* 71: 425-436.
- Johnson, S. C. and Albright, L. J. 1992. Comparative susceptibility and histopathology of the host response of naive Atlantic, chinook, and coho salmon to experimental infection with *Lepeophtheirus salmonis* (Copepoda: Caligidae). *Dis. aquat. Org.* 14: 179-193.
- Johnson, S. C., 1993. A comparison of the development and growth rates of *Lepeophtheirus salmonis* (Copepoda: Caligidae) on Atlantic (*Salmo salar*) and chinook (*Oncorhynchus tshawytscha*) salmon. In: Boxshall, G. A., DeFaye, D. [eds.] *Pathogens of wild and farmed fish: Sea lice*. pp. 68-80.
- Johnson, S. C., Blaylock, R. B., Elphick, J. and Hyatt, K. 1996. Disease caused by the salmon louse *Lepeophtheirus salmonis* (Copepoda: Caligidae) in wild sockeye salmon (*Oncorhynchus nerka*) stocks of Alberni Inlet, British Columbia. *Can. J. Fish. Aquat. Sci.* 53: 2888-2897.
- Johnson S. C. and Fast, M. D. 2004. Interactions between sea lice and their hosts. In: Flik, G., Wiegertjes, and Wendellar-Bonga, S. [eds.] *Host Pathogen Interactions. SEB Symposium Series 55*, 131-160
- Johnson, S. C., Treasurer, J. W., Bravo, S., Nagasawa, K. and Kabata, Z. 2004. A review of the impacts of parasitic copepods on marine aquaculture. *Zool. Stud.* 43: 8-19.
- Jones, S. R. M. and Groman, D. B. 2001. Cohabitation transmission of infectious salmon anaemia virus among freshwater-reared Atlantic salmon. *J. Aquat. Anim. Health* 13: 340-346.
- Jónsdóttir, H., Bron, J. E., Wootten, R. and Turnbull, J. F. 1992. The histopathology associated with the pre-adult and adult stages of *Lepeophtheirus salmonis* on the Atlantic salmon, *Salmo salar* L. *J. Fish Dis.* 15: 521-527.

- 
- Kabata Z. 1972. Developmental stages of *Caligus clemensi* (Copepoda: Caligidae). J. Fish. Res. Bd. Can. 29: 1571-1593.
- Kabata Z. 1974. *Lepeophtheirus cuneifer* sp. nov. (Copepoda: Caligidae), a parasite of fishes from the Pacific coast of North America. J. Fish. Res. Bd. Can.: 31 (1): 43-47.
- Kabata, Z. 1988. Copepoda and Branchiura. In: L. Margolis and Z. Kabata [eds.] Guide to the parasites of fishes of Canada. Part II-Crustacea. Canadian Spec. Pub. Fish. Aquat. Sci.101: 3-127.
- Margolis L. and Arthur, J. R. 1975. Synopsis of the parasites of fishes of Canada. Bull. Fish. Res. Bd. Can. Number 199. Ottawa. 269 pages.
- McDonald T. E. and Margolis, L. 1995. Synopsis of the parasites of fishes of Canada (1978-1993). Canadian Special Publication of Fisheries and Aquatic Sciences No. 122. National Research Council of Canada, Ottawa. 265 pages.
- Øines, Ø. and Heuch, P. A. 2005. Identification of sea louse species of the genus *Caligus* using mtDNA. Journal of the Marine Biological Association of the UK 85: 73-79.
- Parker, R. R. and Margolis, L. 1964. A new species of parasitic copepod, *Caligus clemensi* sp. nov. (Caligoida: Caligidae), from pelagic fishes in the coastal waters of British Columbia. J. Fish. Res. Board Can.: 21: 873-889.
- Pike A. W. and Wadsworth, S.L. 1999. Sea lice on salmonids: their biology and control. Adv. Parasitol. 44: 233-330.
- Schram, T. A. 1993. Supplemental descriptions of the developmental stages of *Lepeophtheirus salmonis* (Krøyer, 1837) (Copepoda: Caligidae). In: *Pathogens of wild and farmed fish: sea lice*. (eds. Boxshall, and Defaye, D.) pp. 30-50. Chichester, Ellis Horwood.
- Schram, T. A. 2004. Practical identification of pelagic sea lice larvae. J. Mar. Biol. Assoc. U.K. 84: 103- 110.
- Tjensvoll, K., Hodneland, k., Nilsen, F. and A. Nylund 2005. Genetic characterization of the mitochondrial DNA from *Lepeophtheirus salmonis* (Crustacea: Copepoda). A new gene organization revealed. Gene 353: 218-230.
- Tucker, C. S., Sommerville, C. and Wootten, R. 2002. Does size really matter? Effects of fish surface area on the settlement and initial survival of *Lepeophtheirus salmonis*, an ectoparasite of Atlantic salmon *Salmo salar*. Dis. Aquat. Org. 49: 145-152.
- Tully, O. and Nolan, D. T. 2002. A review of population biology and host-parasite interactions of the sea louse *Lepeophtheirus salmonis* (Copepoda: Caligidae). Parasitol. 124: 165-S182.
- Tully, O., Poole, W.R., Whelan, K.F. and Merigoux, S. 1993. Parameters and possible causes of epizootics of *Lepeophtheirus salmonis* (Krøyer) infesting sea trout (*Salmo trutta* L.) off the west coast of Ireland. In: *Pathogens of wild and farmed fish: sea lice*. (eds. Boxshall, G.A. and Defaye, D.) pp. 202-218. Chichester, Ellis Horwood.



---

**APPENDIX 2: PROTOCOLS & GUIDELINES**  
**A REFERENCE MANUAL FOR RESEARCH INVOLVING WILD/CULTURED FISH**  
**INTERACTIONS WITH SEA LICE**

**2 - Field Sampling Methods for Juvenile and Adult Pacific Salmon, and Caligid Zooplankton**  
**Compiled by Salmon and Freshwater Ecosystems Division, Science Branch, DFO**

---

- A. INTRODUCTION
  - A.1 Experimental and Sampling Design
  - A.2 Determination of Sample Size
- B. SAMPLING GEARS AND PROTOCOLS FOR JUVENILE PACIFIC SALMONIDS
  - B.1 Beach Seining
  - B.2 Purse Seining
  - B.3 Trawling
  - B.4 Trolling
- C. PROCESSING OF WILD FISH IN LABORATORY AND AT SEA, WATER PROPERTY SAMPLING, AND DATA PROCESSING
  - C.1 Processing of wild fish in the laboratory
  - C.2. Processing of wild fish at sea.
  - C.3 Water property sampling
  - C.4 Field data processing
- D. CALIGID ZOOPLANKTON SAMPLING
  - D.1 Background on motile stages of Caligids
  - D.2 Sampling design considerations
  - D.3 Zooplankton Sampling Methodology
  - D.4 Sampling design considerations
  - D.5. Preservation of zooplankton samples
- E. SAMPLING ADULT SALMON: ESCAPEMENT ESTIMATION
  - E.1 Standards
  - E.2 Protocols and guidelines
  - E.3 Escapement Estimation Methodology for Pink salmon (an example)
- F. REFERENCES

P&G 2 ADDENDUM 1: TRAWL SURVEY DESIGN

ADDENDUM 2: A BIBLIOGRAPHY OF SURVEY METHODS FOR SALMON ESCAPEMENT  
ENUMERATION

---

---

## A. INTRODUCTION

This chapter will primarily describe sampling techniques recently used by the Department of Fisheries and Oceans in studies of juvenile salmon, sea lice, and spawning populations of adult Pacific salmon. Reference will also be made to some additional sampling techniques that have been used by non-Department researchers. **The material presented on sampling techniques is not intended to limit the creative development of other sampling techniques that may best suit different questions and/or environments.** When initiating a sampling program, the tools applied should be those best suited to address objectives defined for the study (e.g., preliminary observation, trend analyses, or quantitative estimation) and the physical environment that must be sampled; and be logistically realistic for the capability of the program staff, funding and equipment available, and access or proximity to the sampling sites. Most sampling of biological processes will be informative but caution is necessary when the results of a sampling program are extended to a broader topic or group of animals beyond those in actual samples collected (i.e., the inferences drawn). All sampling programs will have their limitations, biases, and uncertainties. A thorough sampling program should assess these latter concerns; examine alternative sampling techniques, and appropriately qualify inferences and conclusions drawn.

### A.1 Experimental and Sampling Design

There are many reasons why a sampling program may be developed, including:

- preliminary data collection and examination (distribution of fish in time and space, monitoring growth and health of the fish, estimation of sample variances, etc.),
- estimating the abundance of a species in a defined area (sampling within a structure design for extrapolation to total abundance),
- comparative trend analyses within a population over time (for example, annual evaluations of spawning escapements in Pacific salmon populations), or
- testing experimental hypotheses or sampling for validation of an analytical model.

The need for a structured sampling design will vary with the goals of a program but all sampling programs should consider basic guidelines for experimental design. Two classes of experiments are typically identified: mensurative (taking measurements at one or more points in time and space where time and space are the “experimental” variables) and manipulative (involving treatments or imposition of external factors). In ecological studies though these distinctions can become confounded and challenge the researcher to develop logical designs that strengthen conclusions drawn from the experiment. The topic of experimental design and sampling in ecological settings is much larger than this Chapter can address but there are useful texts for reference (Green 1979, Krebs 1999, Quinn and Keough 2002, Sokal and Rohlf 1995) and an extensive scientific literature (e.g., Eberhardt and Thomas 1991, Hurlbert 1984, 2004; Heffner et al. 1996; Carpenter and Matson 1990; Okansen 2001, 2004)<sup>1</sup>. Appendix 2, by Karin Boxaspen, also contains brief information on some Norwegian approaches with references in the general bibliography.

The challenges in ecological studies were well described in Hurlbert’s (1984) paper. Hurlbert describes four fundamental features in experiment design: controls (i.e., baseline comparisons or reference sites), replication of measurement to control for stochastic events, randomization of treatment or sites to control for experimental error or bias, and interspersing of treatment or sites to control for unforeseen time and/or area effects.

---

<sup>1</sup> These references are only a small fraction of the scientific literature. Web searches for “experimental design” or “sampling in ecology” will provide many citations to different species, environments, and tasks.

---

“An experiment is successful to the extent that these factors are prevented from rendering its results inconclusive or ambiguous. It is the task of experimental design to reduce or eliminate the influence of those sources [sources of confusion Table 5.2] ...”<sup>2</sup>

A researcher planning an experiment or sampling program in an ecological setting is confronted with the difficult task of addressing these classical features of design. The researcher would need to define the experimental or sampling unit (what is being sampled?), randomize treatments and references, replication, and assessing interspersion of treatments and reference sites over time and space. In ecological situations though, the researcher is often not free to randomize treatments, the number of replicates may be limited, and results within an area are rarely independent of each other. Given these limitations, the researcher should consider three questions:

1. What response variable can be measured, how to compare samples or treatments, and how to estimate the variability in that response variable (sample sizes and variances)?
2. Is strict adherence to empirically-based statistical inference necessary, and if not, then what evidence would be adequate for comparison of treatments?
3. Would other experimental designs be more appropriate to the study?

The latter issue may involve adaptive management designs or temporal interventions (see Walters 1986, Walters et al. 1988) or designs presented by Oksanen (2001). Oksanen specifically addressed concerns of “pseudoreplication” identified by Hurlbert (1984) and suggests three designs that may suit ecological studies: use of predictions which are testable and address system dynamics, comparing a single treatment with replicated controls, and conducting an un-replicated experiment without application of inferential statistics. If an experiment is not replicated, there is no possibility to statistically establish a connection between treatment and the apparent effect<sup>3</sup>. When randomization and interspersion cannot be fully implemented in large observational ecological studies, it remains important to replicate studies (with the best reference[s] possible) in multiple locations. Such “replicates” do not lend themselves to standard statistical analyses and care must be taken in what inferences are drawn, but information can be gained by careful attention to standardization of methods and data collection procedures. Further, if a study involves a ‘before-and-after’ contrast (i.e., an intervention, such as a fallowing period), the review by Michener (1997) provides some statistical approaches for the analysis of such data.

Consideration of experimental design should receive greater attention than it generally does, in order to “account” for the challenges of sampling large open ecological systems with inherently variable natural systems (involving multiple interacting environmental factors); and/or monitoring populations over long timeframes with the expected changes in environments through time (time treatment interactions). Sampling programs in natural systems may require large financial investments and potentially have significant impacts for resource management and policy development. While consideration of program objectives and statistical design are strongly recommended before any sampling program is initiated; the design must be realistic given the environmental conditions involved and logistic limitations to the study. Logistic realities are critical to the design of an effective study or sampling program. A successful program is as much a function of successful execution of a design as it is about developing a design. A complex but unrealistic design may have the potential for providing greater resolution or clarity, but may also have a high risk of failing to meet the design needs and providing inaccurate and inconsistent information. Balancing the objectives of a sampling program (inferences to be drawn and potential impact of the results) with the logistics to successfully

---

<sup>2</sup> Hurlbert’s (1984) Table 5.2 provides a useful summary of the sources of confusion in experiments and the role of these four features in experimental design.

<sup>3</sup> Pseudoreplication is defined by Hurlbert (1984) as the use of inferential statistics to test for treatment effects with data from an experiment where either treatments were not replicated or experimental units are not statistically independent.

---

implement the program and with cost-effectiveness can really only be usefully considered on a case-by-case basis.

## A.2 Determination of Sample Size

In order to determine the number of samples needed to answer a particular research question, some key information is required. First, what is the statistical hypothesis to be tested? The necessary calculations to determine the necessary sample size are dependent on the nature of the question (see Neter et al. 1996 for examples such as unequal sample sizes, use of Power Tables, etc.). In this report, the interest may be in testing for differences between mean abundances of lice from fish sampled at different locations. To determine the number of fish to be sampled, the sample size formula for this question is:

$$n \geq 2 \left( \frac{\sigma}{\delta} \right)^2 \left\{ t_{\alpha[u]} + t_{2(1-P)[u]} \right\}^2 \quad (\text{Sokal and Rohlf 1981})$$

To determine the sample size information is needed on an estimate of the sample variability ( $\sigma$ ), the desired detectable difference ( $\delta$ ), the significance level ( $\alpha$ ) and the intended power of the test ( $P$ ). (Note that “power” is the ability of the test to detect a statistically significant difference when it truly exists.) Sample variability is usually found through prior analyses, test samples, or previously published results. As in the t-test or any ANOVA,  $\sigma$  is assumed equal over all groups to be tested. The other missing elements are commonly decided by resolving the conflict between obtaining ideal results and the limiting financial or logistical realities of the study (obtaining more observations will almost always lead to more informative results, but often at prohibitively expensive costs of time, money and resources). Quite often, the merits of several “scenarios” are weighed prior to choosing a “best case” combination of sample size, power, significance and detectable difference.

Intuitively, the formula states that fewer samples per group (smaller  $n$ ) will be required to detect coarse differences between well-defined populations (i.e. when the variability  $\sigma$  is small relative to detectable difference  $\delta$  that is sought, resulting in a small ratio  $\sigma/\delta$ ). Conversely, it becomes increasingly difficult (and thus, requires larger  $n$ ) to detect small differences between populations with high variability (i.e.  $\sigma/\delta$  is large). Both  $\sigma$  and  $\delta$  can be expressed in percentages of the means, and thus be expressed as a ratio instead of knowing their values in absolute terms.

The solution to this formula is found through iteration. It starts with an initial “guess” of an appropriate  $n$  to find the degrees of freedom for the values of the t-distribution,  $t_{\alpha[u]}$  and  $t_{2(1-P)[u]}$ . It is important to note that  $n$  refers to the number of samples *per group*, not in total, for the study. Thus, the degrees of freedom ( $u$ ) of the initial t-values (at probability levels  $\alpha$  and  $[1-P]$ ) are defined as  $u = \text{number of groups in the study multiplied by } (n^*-1)$ , where  $n^*$  is the initial guess. The resulting  $n$  is then used to update the t-values in the formula and this process is repeated until convergence on an ideal  $n$  is achieved.

## B. SAMPLING GEARS AND PROTOCOLS FOR JUVENILE PACIFIC SALMONIDS

The following descriptions only involve gears presently used by DFO in studies to sample juvenile Pacific salmon and monitor sea lice infection levels. Readers should note that there are other sources of sampling protocols for Pacific salmon studies that could also provide useful reference materials, for example:

[Washington department of fish & Wildlife, fishing & Shellfishing](#)

[Research, Monitoring & Reporting, British Columbia Environment](#)

Two sampling gears that are not described but have been applied in British Columbia are dip nets (Morton and Williams 2003, Morton et al. 2004) and ocean fish-lift trawls developed in Norway (Holst et al. 1993) and adopted to coastal sampling by Dr. Allen Gottesfeld (Skeena Fisheries Commission)

---

and Mr. Dave Rolston. More information on Norwegian methods can also be found in Appendix 2 by Karin Boxaspen.

A comparison of the sampling gear is provided in Table 1. Dip nets were used in the Broughton Archipelago to collect the first samples of juvenile pink salmon observed with extensive numbers of sea lice (Morton and Williams 2003). Dip nets collect small numbers of fish but the small area sampled and concern for the size or health of fish caught has generated concern about how representative the collection would be of the population. A sample collected by dip net can be informative but exemplifies the need for care in what inferences are drawn based on those samples.

The gears described present a sequence of sampling techniques necessary for sampling juvenile pink and chum salmon as they utilize the near-shore marine habitats during their first few months. Initially pink and chum utilize the shallow margins of the marine habitat and may be highly abundant and accessible to beach seine gear. As the animals grow they will begin to use deeper waters and require the use of purse seines and then trawl gear to access a broader range of body sizes. The literature on pink and chum salmon suggests they begin to move into deeper waters when they attain 5 to 6 cm in body size. Given this behaviour, each of these sampling gears may be necessary to understand fully the production dynamics and coastal habitat use in these species.

### **B.1 Beach Seining**

Beach seining is a fishing method that is commonly used to capture juvenile salmon in fresh water, and, during the early sea life period, soon after the young salmon enter the marine environment. During the early sea life period juvenile pink and chum salmon are typically concentrated in shallow water close to shore. The depth of the water is frequently too shallow for these fish to be captured efficiently with a purse seine, but beach seining can be effective.

A beach seine is simply a fishing net that is designed to be fished along the shore, to capture fish that are found in very shallow water (e.g. <5 meters depth). The web of the net is suspended between numerous floats attached along the top edge of the net, and a heavier lead-filled line attached along the bottom edge of the net. Beach seine nets can be constructed in many different configurations, depending on the requirements. The three main defining characteristics are the overall length of the net, the depth of the net, and the mesh size(s) of the web material that are used. Nets used for beach seining are usually custom designed and built for capturing the target fish species. Beach seines are usually fished manually so keeping the net as short and shallow as possible, and using the largest possible mesh size that will retain all of the target fish species that are likely to be encountered will reduce the amount of effort required to fish the net.

Beach seine nets used to capture juvenile salmon are typically between 15 and 60 m in length and 1.5 to 6 m deep. The mesh size of the web that is used depends on the size of the target salmon species. For example, the beach seine nets used in the DFO program to sample juvenile pink and chum salmon in the Broughton and in Knight Inlet from 2003–2005 were 46 m long and 3.7 m deep. These nets were constructed of three 15.2 m long panels sewn together. The web in the 15.2 m long panels at both ends of the net had a stretched mesh size of 1.27 cm. The middle 15.2 m long “bunt” panel of each net had a stretched web size of 0.64 cm. The larger mesh size of the web used on both ends of the net allowed the net to be hauled through the water with less physical effort. The smaller mesh size of the web in the middle section of the net ensured that even the smallest pink salmon were retained as the captured fish were finally crowded into the bunt section of the net. A lead-filled rope (lead-line), weighing 0.5 kg per meter of length, was attached to the bottom edge of



*Figure 1. Beach seining Broughton Archipelago.*

---

the net. Corks (99 total) were attached at regular intervals to the cork line attached to the top edge of the net, which provided sufficient buoyancy to float the entire net under all water current and weather conditions.

Fishing with a beach seine is commonly done by three people wearing chest waders, and using a small open boat. For example, Boston Whaler open console "Montauk" boats, 5.5 m in length) and powered by a single 70 or 90 horsepower outboard motor with hydraulic lift on the motor leg, have been used extensively by DFO and have proven to be suitable for beach seining.

Table 1. Comparison of sampling gear.

Gear	Sample Areas	Target Species/Fish Size	Vessel & Personnel Requirements	Application/Benefits	Problems
Beach seine (DFO protocol)	Nearshore areas (< 5 m)	- Juvenile salmon, - Other nearshore species	- Small boat capable of working nearshore (eg. Boston Whaler) - 3 people	1. Can sample shallow water areas. 2. Can sample small fish. 3. Gear costs low.	1. Sampling limited to nearshore and may not be representative of populations with wider distribution. 2. Some loss or transfer of motile sea lice could occur.
Purse seine (DFO protocol)	Surface waters in nearshore and open ocean areas (> 5 m depth)	- All age classes of Pacific salmon. - Other pelagic fish species.	- Medium to large vessel (> 10 m) with specialized gear. - 4-5 people	1. Can sample surface waters > 5 m 2. Can sample all age classes of fish.	1. Cannot sample waters < 5 m. 2. Limited to sampling surface waters. 3. Difficult to fish in high current areas. 4. Some loss or transfer of motile sea lice could occur.
Trawling (DFO protocol)	Waters > 25 m	- All age classes of Pacific salmon - Other pelagic and demersal fish species	- Large vessel with specialized gear and hp to tow gear. - 10+ people	1. Can sample all water column depths. 2. Samples large volume of water over short time period (~2 x 10 <sup>6</sup> m <sup>3</sup> water in 30 minutes). 3. Can be used to estimate abundance of species.	1. Limited to waters > 25 m. 2. Some sea lice likely to be lost or transferred while in net. Data not used to enumerate sea lice levels. 3. Cannot fish in restricted or confined areas. 4. High cost.
Trolling (DFO protocol)	Waters > 5 m	- Salmon >25 cm - some rockfish species	- Medium sized vessel equipped with specialized gear - 3 people	1. Can sample fish individually with minimal sea lice loss and no lice transfer. 2. Can fish most locations > 5 m.	1. Lower sample size per day. 2. Moderate cost
Dipnets	Surface waters	- Juvenile salmon	- Small vessel - 1 person	1. Can sample individual fish. 2. Low cost	1. Sample not representative of population. Tend to get poor performers or fish that are not schooling.
Ocean fish-lift trawl	Surface waters	- Juvenile salmon - Other pelagic species	- Medium size vessel	1. Can sample surface waters 2. Reduced gear impact on salmon compared to trawl.	1. Some transfer of motile sea lice could occur. 2. High cost for special gear.

---

To capture wild fish with a beach seine the net is loosely piled by hand into the bow of the boat. At each fishing location the operator steers the boat slowly, bow first, into the shore until a second person can safely step ashore or into shallow water. This second person pulls one end of the beach seine net ashore and then holds fast this end of the net. Alternatively, if fewer people are available then this end of the net can be tied to the shore. The boat operator then slowly backs up the boat away from the shore, first perpendicular from the shore and then parallel to the shore. As the boat moves along the beach the rest of the net gradually comes off the bow of the boat, usually without any assistance required from the people in the boat. As the last part of the net goes into the water, the boat operator sharply turns the bow of the boat towards the shore and then slowly steers the boat directly into the shore until the third person can safely step ashore or into shallow water. This third person then pulls ashore the end of a long rope that is attached to second end of the beach seine net. The boat is then free of the net and is moved away from the net to ensure the propeller does not get caught in the net. The third person then commences to pull on the long rope (c.a. 15 m long) until the second end of the net comes ashore. Both people on the shore then slowly move closer together along the shore while also continuing to steadily pull on their end of the net. Once the two ends of the net are close together (about 2-3 m apart) most of the net is gradually pulled in by hand and piled on the shore or in very shallow water. During this operation both the cork line and lead line are pulled simultaneously and equally on each side of the net. This eventually crowds any fish caught in the net into the centre “bunt” portion of the net which typically has the smallest size mesh. As the last roughly 10-15 meters of the net is pulled into the shore the lead line is pulled slightly faster than the cork line. This results finally in the last section of the lead line coming out of the water while some of the net and cork line still remains in the water. When done correctly this caused the net to form a bag in shallow water in which all of the fish are retained and enclosed by the net, but the fish still remain fully submerged in water. All of the fish captured can then be removed from the net and then retained, sampled or released. After the catch is removed the net is manually piled back onto the bow of the boat and is ready for the next fishing operation.

The term “beach seine” may be misleading to people who are not familiar with this fishing method. A beach seine is easiest to use in locations that have a gently sloping beach, with a smooth sand or mud bottom. However, with sufficient experience, skill and determination it is possible to capture wild juvenile salmon using beach seines at locations that commonly would not be recognized as a “beach”, including uneven and rocky shores, and in some cases steep shores or even sheer cliffs. Similar comments and descriptions were provided by Ms J. Osborne concerning beach seine sampling in Clayoquot Sound, west coast of Vancouver Island. Details of their field methods for beach seining juvenile salmonids are provided in:

*Osborne, J. 2005. Clayoquot Sound Sea Lice Working Group. 2004 Interim Data Report. Report to the Westcoast Aquatic Management Association and the BC Innovation Council. 15 pp + appendices.*

## **B.2 Purse Seining**

A purse seine is a fishing net that is designed to capture pelagic fish species that are found close to the surface in either fresh water or the ocean. Purse seining is a standard fishing method that is widely used in both commercial fisheries and in scientific surveys to capture juvenile or adult stages of many pelagic fish species. When used to sample Pacific salmon, purse seining is typically used in near-shore and coastal areas and can be an effective method for capturing both juvenile and adult salmon when these fish are located in deeper water (depth > 5 m), but still relatively close to shore. However, purse seining has also been used extensively and successfully to capture both juveniles and adults of all five species of Pacific salmon in the open ocean (Hartt 1966; Pearcy and Fisher 1990).





A simplified description of a purse seine is essentially a fishing net that is suspended between numerous floats attached along the top edge of the net, and a heavy lead-filled line attached along the bottom edge of the net. Purse seine nets can be constructed in many different configurations, depending on the requirements. The three main defining characteristics are the overall length of the net, the depth of the net, and the mesh size(s) of the web material that are used. Additional important specifications, which will depend on the final length, depth and weight of the mesh used to construct the net, include the number and size of the corks used to float the net, and the weight of the lead line.

To capture wild fish with a purse seine the net is usually deployed from a fishing vessel. The fishing vessel releases one end of the net into the water and then eventually tows the other end of the net into the shape of a complete circle. At this stage the net has enclosed a large volume of water within the circle of the net, but the bottom of the net is still open. The open bottom of the net is then completely closed off by using a hydraulic-powered purse winch to gradually tighten a purse line, which is a free running rope that passes through metal rings that are attached at frequent intervals near the bottom edge of the net. After the bottom of the net is closed off the net is ideally shaped like a cup or “purse” and hence the origin of the name “purse seining”. The net is then gradually spooled back onto the net drum until only a small portion of the net (known as the “bunt”) still remains in the water alongside the vessel. This procedure gradually forces any fish caught inside the net to be crowded into a smaller and smaller amount of water close to the side of the vessel. Finally almost all the net is back aboard the vessel and the captured fish are all concentrated into the bunt section of the net, close to the side of the fishing vessel. The fish are then removed from the bunt, either by hauling the entire bunt aboard the vessel, or by brailing or pumping the fish out of the bunt and onto the vessel. All of the fish can either be retained (e.g. in commercial fisheries) or only a sample can be taken and the rest of the fish released (e.g. in research programs).

A wide variety of purse seine configurations have been used to sample wild Pacific salmon. For example, the DFO surveys of juvenile salmon and sea lice conducted in the Broughton Archipelago during 2003- 2005, used custom-made purse seine nets. The overall length of one net was 178 meters, with a depth of 13 meters. The stretched mesh size of the web in the lead section of this net was 3.2 cm and in the bunt section was 1.9 cm. To ensure even the smallest sizes of pink salmon were captured, a liner panel of smaller mesh was attached inside the bunt section only and had a stretched mesh size of 0.63 cm.

Purse seines can be fished in various ways, with the two most common methods being “circle sets” and “shore tie-up sets”. To capture fish in a “circle set” the fishing vessel slowly moves forward in a circle pattern while the net is simultaneously released into the water. The vessel circles completely around and when it reaches the end of the net that first entered the water, the free end of the net is secured to the side of the vessel. In circle sets the net is usually “closed up” and then pursed immediately after setting the net. In other words the net is set and closed up into a complete circle in one continuous procedure, and is not held open to allow time for more fish to swim into the net before the circle was closed.

A common alternative to the “circle set” is known as a “shore tie-up” set. For this type of set a rope attached to the leading end of the purse seine is taken ashore using a seine skiff and secured to a convenient location (e.g. rock, tree, steel “eye” set into the rock, etc.). The seine vessel then

---

releases the purse seine net into the water as the vessel moves away from the shore. The vessel is usually manoeuvred so that when all of the net is in the water the net is arc-shaped, with the concave side of the arc facing into the prevailing water current. The net is then typically held open in this arc shape for a specified period of time (e.g. 30 minutes). The fishing vessel then pulls the end of the net that is still attached to the vessel back towards the shore. As the seine vessel reaches the other end of the net that is still tied to the shore, the shore line is released and the net is then fully closed up into a complete circle. The bottom of the net is again then closed up using the purse line.

There are several advantages and disadvantages to this “shore tie up” method of purse seining. The main advantage is that this can permit purse seining in locations where the tidal currents or winds are very strong and circle sets are not possible (e.g. the fishing vessel would be carried too far by the currents or wind during the set). A second advantage is that when the tidal currents are strong a much larger volume of water passes through the seine net, compared to a circle set. This may allow capture of larger numbers of fish, particularly if the fish are either being carried passively, or are actively swimming, in the same direction as the tidal currents. Some researchers have also used several shore tie-off sets made at the same location, but with the “open” (concave shaped) side of the net facing in opposite directions, to determine the direction of migration of fish. Two of the main disadvantages of the shore tie off method are that:

- 1) it is much more dangerous due to the requirements to tie the end of the net to the shore and then subsequently release the shore end of this rope while it is still under extreme tension; and,
- 2) the volume of water that is sampled by the net typically cannot be reliably determined or standardized, which may compromise any estimates of abundance of the fish that are captured at other times or locations.

The size of the fishing vessel required for purse seining depends mainly on the size of the purse seine net, and the wind and current conditions that the vessel must operate in. On modern purse seine vessels used in B.C., the purse seine net is usually stored on a large hydraulically-rotated “drum” (spool) near the stern of the fishing vessel. The drum must be large enough to hold the entire net, and the hydraulics system on the vessel must be powerful enough to bring the net back onto the drum under all sea and weather conditions expected to be encountered in the fishing area. The weight and volume of a purse seine net can be large, especially when the net is wet. A large catch of fish can also exert tremendous forces on both the net and the vessel, sometimes sufficient to affect the stability of the vessel. So the fishing vessel must be large enough and properly designed to ensure the additional weight of the wet net when it is on the drum, and the additional forces exerted by the weight or behaviour of the fish in the net, do not dangerously compromise the safety or stability of the vessel.

Adult fish of all five species of Pacific salmon are typically captured using purse seine nets and fishing vessels that are used in commercial salmon fisheries. Purse seine vessels used in the commercial salmon fleet in B.C. are typically in the range of 18 to 27 m in length. The cost of a new purse seine net used for commercial salmon fisheries in B.C. can range from about Can\$45,000 to \$70,000. Several different configurations of commercial purse seine nets are currently used in B.C., depending mainly on the time of year, the geographic area and the target fish species. The main dimensions of purse seine nets that fishermen are permitted to use in commercial salmon fisheries in B.C. are specified in the [Pacific Fisheries Regulations, 1993](#) of the Canadian federal *Fisheries Act*.

The relevant section states:

- 60 (1). For the purposes of this section, "depth", in respect of a purse seine, means the sum total of the mesh sizes of all meshes in a perpendicular row of meshes from the corkline to the lead line; (*chute*)

- 
- "length", in respect of a purse seine, means the aggregate total length of the seine corkline and any lead attached to it and includes any part of the seine corkline on the net drum of the vessel from which it is being used. (*longueur*)
  - 60(2). No person shall fish for salmon with a purse seine that
    - (a) has a mesh size of less than 70 mm;
    - (b) is less than 270 m in length; or
    - (c) is less than 20 m in depth.
  - 60(3). No person shall fish for salmon in any Area other than Area 20 with a purse seine that is more than
    - (a) 400 m in length; or
    - (b) 52 m in depth.
  - 60(4). No person shall fish for salmon in Area 20 with a purse seine that is more than
    - (a) 550 m in length; or
    - (b) 80 m in depth.

Purse seine nets used to capture wild juvenile salmon are typically smaller than commercial purse seine nets, and are custom designed and constructed. Juvenile Pacific salmon of all species commonly are located closer to shore, and in shallower water, than adult salmon. The purse seine nets used to capture adult salmon are typically too deep (and often too long) to capture juveniles effectively in these locations. Although smaller purse seine nets can be still fished by a large commercial fishing vessel, the reduced size and weight of the smaller purse seine nets required to sample juvenile salmon may allow a smaller vessel to be used. Using both a smaller seine net and a smaller fishing vessel usually results in smaller fish catches, but also lower overall operating costs. These can be major advantages for many scientific surveys where the main requirements are typically to capture only small numbers of fish (to obtain representative samples of fish populations) and also to minimize costs.

Several different fishing vessels were used in the purse seine sampling of juvenile salmon in the DFO surveys conducted in the Broughton and Knight Inlet from 2003-2005. In the Broughton the purse seining in 2003 and 2004 was conducted using the DFO purse seine vessel "Walker Rock". This vessel is 13.5 m in length, constructed of welded aluminium, and was custom built in 1970 for DFO research on salmon.

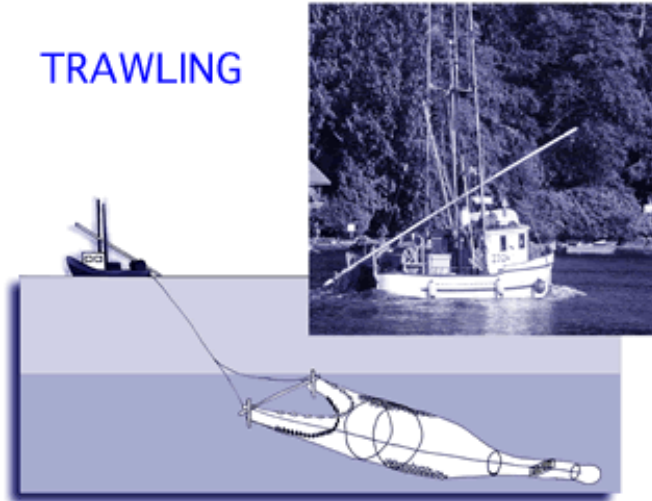
In 2003 the purse seining in Knight Inlet was conducted by three commercial salmon purse seine vessels (Cape Lazo, Western Eagle and ALH), ranging in size from 20 to 23 m in length, that were contracted by DFO specifically for this work. These larger vessels were more suitable for purse seining in the rougher sea conditions and stronger winds and tidal currents of Knight Inlet, compared to the Broughton.

---

### B.3 Trawling



#### TRAWLING



Dr. R. Beamish and staff have provided extensive details on trawl survey design and statistical methods used in estimating total abundances based on results of the trawl sampling. This material is informative for those interested in the development of trawl surveys but is substantially more detailed than other materials provided for Chapter 2. The material was therefore provided in Addendum 1 to this chapter.

The survey design, the type of net, and the method of fishing enables a researcher to catch all sizes of Pacific salmon, at any depth, during virtually all weather conditions in the Strait of Georgia. The mid-water rope trawl used by DFO Science has an opening approximately 15 m by 30 m, and approximately  $2.08 \times 10^6 \text{ m}^3$  of water are filtered by the trawl during an average 30 minute tow at a speed of approximately 5 knots per hour. It is assumed that all fish in front of the net were captured, although it is probable that the some of the fish were able to avoid the net (i.e., catchability of the net is less than one).

However, the estimated abundances were assumed to be consistent relative values (indices) within and among years. The catches and abundance estimates, therefore, are measures of the population dynamics of juvenile Pacific salmon in the same way that catches in standardized research surveys are a common tool in stock assessment (Doubleday and Rivard 1981). Abundance was estimated using the method initially developed in Beamish et al. (2000) and now extended and described in Addendum 1 attached to this chapter.

It is important to note that trawl sampling, without a live capture box, will remove some scales and sea lice on the capture fish. Without a comparative sampling method to adjust for this expected effect, inferences drawn from trawl sampling must recognize these limitations.

---

## TROLLING



### B.4 Trolling

Sampling sea lice on salmon is a difficult task as the mobile stages of sea lice of all species are able to move around. In any sample of salmon (trawl, seine etc) mobile sea lice could be either lost or transferred to other salmon in the sample. Trolling is the only sampling method that will eliminate the risk of transfer of sea lice between salmon in a sample. The following procedure was used to sample age 0+ juveniles, immature and adult salmon using commercial troll gear (Beamish et al. 2005).

A commercial troller was used to sample wild Pacific salmon although hook and line gear (i.e., rod and hook) can also be used for other species

or confined areas (e.g. inside net pens). With the commercial troll samples, trolling speed is determined by the expertise of the commercial fisherman and by species of salmon being targeted. The troll gear used for sampling can be mixed depending on species of salmon being targeted, time of year, size of salmon and reports from sport or commercial fishermen on what is working in the sample area. Gear that has been used includes flashers (rectangular metal plates used to attract Pacific salmon) and a variety of hook sizes, lure colours and types. Fishing is conducted at multiple depths throughout the water column. Fishing effort is reduced if the number of fish being caught exceeds the sampling personnel's ability to process them. When trolling, various species of salmon may be caught concurrently, therefore, when a target sample size for any given species is achieved, subsequent catches of that species are released.

Sampling using troll gear has a number of notable advantages in studies of juvenile salmonids. This method of sampling is effective on salmon greater than 25cm (approximately September of first year at sea) and an experienced troller can be selective (to some degree) on the species and size of salmon being sampled in most areas and times of year. Trolling permits samples to be collected over both a variety of areas and depths and permits multiple species to be sampled concurrently without risk of cross infection of sea lice. In selecting a troll vessel for sampling, adequate deck space to conduct sampling is an important consideration.

## C. PROCESSING OF WILD FISH IN LABORATORY AND AT SEA, WATER PROPERTY SAMPLING, AND DATA PROCESSING

The procedures used to process wild juvenile salmon in the lab or at sea that were captured during the DFO beach and purse seine surveys to assess sea lice infections in the Broughton during 2003-2005 were simple. These procedures are described in sections C.1 and C.2 below, and describe current practice. Note also that live sampling of juvenile salmon has recently been described by Krkosek et al. 2005.

### C.1 Processing of wild fish in the laboratory

Fish that are captured with either a purse seine or beach seine are placed into individual Whirl-Pak<sup>®</sup> or Zip-Lock<sup>®</sup> bags directly from the bunt of the nets. Once the net dries up sufficiently to concentrate the fish in the bunt, samples of up to 30 fish of each species are removed directly from the bunt, one fish at a time. Each individual fish is captured alive and free-swimming from the bunt and placed into an individual sample bag. Care should be taken to avoid or minimize handling. The fish that are individually bagged are also chosen as randomly as possible from the entire catch in the net. However, it should be recognized that the sampling procedure that has been used is not truly random. For example, when catches are large a truly random sampling

---

protocol might involve removing all fish caught, one fish at a time, and retaining only every 10<sup>th</sup> fish. This rigorous sampling approach simply was not practical for the DFO surveys in the Broughton due to the large numbers of fish that frequently were caught and the number of sets in one survey.

Placing each fish into an individual plastic bag typically results in some amount of seawater also being enclosed in the bag. To minimize the time required to freeze the fish each bag can be punctured several times with a sharp needle to allow the seawater to drain out of the bag. The diameter of the needles should be much smaller than the size of even the smallest motile sea lice, to ensure no sea lice that might fall off the fish could be lost through the holes in the bags. The non-motile stages of sea lice are smaller (down to microscopic size) but are firmly attached to the fish with a strong filament. It is therefore assumed that these younger and smaller stages of sea lice should remain attached to the fish, and therefore not be lost through these holes in the bags.

The samples of fish that are retained in plastic bags are labelled and immediately placed in either 12 volt DC or 120 volt AC chest freezers. These samples remain stored in the freezers aboard the fishing vessels until the end of each field survey (7-10 days). The fish samples are then transferred from the freezers on the fishing vessels into large "Coleman" coolers equipped with pre-frozen "freezer packs". These coolers are typically then taken by truck from Port McNeill to the DFO Pacific Biological Station (PBS) in Nanaimo, B.C., where the fish samples are transferred from the coolers into large plastic garbage bags and then stored in a walk-in freezer at -20 ° Celsius. These fish samples are subsequently analyzed for sea lice in the laboratory at PBS.

When the total catches of fish from each set are small (e.g. less than 300 fish per species), the samples of 30 fish per species are immediately bagged and then all of the remaining fish from each beach or purse seine set are identified by species, counted and released. When the catches were larger (e.g. 300- 500) typically one or two people bag fish, while the other person(s) simultaneously count, identify and release fish from the bunt. For very large catches (e.g. >500), fish are also removed from the bunt using dipnets, rather than individually. In these cases, the total number of dipnets of fish that are removed are counted, and all the fish in several dipnets that are randomly chosen (e.g. every fourth dipnet of fish if there were 12 dipnets of fish in total) and all the fish are placed in five gallon white buckets. After all of the fish in the bunt have been removed, the fish in these buckets are individually counted and identified. The total numbers of fish of each species that were originally captured in the net are then estimated by multiplying the total number of dipnets of fish that were removed from the bunt by the average number of fish of each species in the dipnet samples that have been retained in the white buckets. In some cases in the DFO studies the catches of herring were very large and it was impractical to count every fish, or even by dipnetting the fish out of the bunt. In these cases the catch of herring by weight (tonnes) was estimated visually by the purse seine vessel skipper, and this weight was subsequently converted to number of fish by dividing the weight in tonnes by the average weight per fish.

## **C.2. Processing of wild fish at sea.**

Salmon caught on troll gear are taken off the hook without handling and landed directly into large plastic tubs. These tubs are solid and therefore any sea lice that become dislodged from the salmon are retained within the tub. Only one salmon is placed into any single tub. The salmon is killed with a blow to the head. The salmon are individually examined at sea for sea lice. The salmon is removed from the tub and placed on a sampling board. Salmon are examined by segments: the left side examined first followed by the right side, followed by both the dorsal and ventral surfaces, and all fins. This standardized method of examination reduces the risk of overlooking a segment and sea lice. The numbers of chalimus and of mobile stages are recorded, noting the approximate location on the body of the salmon. Sea lice are removed and preserved in a 30 ml glass vial with 70% ethanol for subsequent identification in the laboratory. Each salmon has a new vial and the vial is labelled with the date and salmon number. For attached chalimus

stages, a small piece of fin, a scale or a piece of flesh is cut out with the louse to ensure it is not damaged. Following removal of sea lice the fish is examined again using 10 X magnification to ensure that all sea lice have been counted. The tub that the salmon was landed in is examined for sea lice and these loose lice are recorded and preserved and the tub is washed clean. The board that the salmon is held on for examination is examined for loose lice and if there are any lice, these are recorded and preserved. The sampling board is washed clean prior to examination of the next salmon. In addition to recording the numbers of sea lice present on each salmon, the amount of scale loss is estimated as a percentage of total area on each side of the fish and damage to the skin is identified using the criteria in Table 2. The level of skin damage is recorded in whole numbers. Therefore, an average skin damage value of 0.5 would indicate that most fish had a skin damage of either 0 or 1. Each salmon is given an individual sample number and along with the sea lice information above, the species, length, weight and sex of the salmon is recorded.

Preserved sea lice samples are returned to the laboratory and identified by experts using the criteria described by Kabata (1972, 1973) and by Johnson and Albright (1991). Sea lice numbers are identified according to the general developments stages of copepodid, chalimus, preadult, adult male, adult female and gravid female. The preadult, adult male and female, and the gravid female stages are also reported as mobile stages. The term prevalence is used to indicate the percentage of fish that were infected and the term intensity is used to identify the number of sea lice per infected fish (Margolis et al. 1982). The term mobile refers to all post-chalimus stages. The stages of sea lice used are consistent with the descriptions of Johnson and Albright (1991).

*Table 2. Criteria used to classify skin damage on Pacific salmon.*

0	No skin damage and no red discoloration of skin surfaces from haemorrhaging.
1	Minor red discoloration from haemorrhaging, but reduced in intensity and in area; no scale abrasion.
2	Moderate haemorrhaging resulting in more red color over an area about one half the size of the anal fin; minor scale abrasion may be present.
3	Severe haemorrhaging, area of haemorrhaging approximately the size of the anal fin or larger and almost uniformly red; no lesions; scale abrasion common, but skin intact.
4	Lesions present, skin removed and muscle exposed or skin partially removed exposing necrotic tissue; haemorrhaging at margins of lesions.

### **C.3 Water property sampling**

Temperature and salinity are known to affect sea lice development and survival rates (Tucker et al., 2000) so in many studies water measurements or samples are collected to assess the salinity and temperature of the water in which the juvenile salmon are captured. Continuous vertical profiles of water temperature and salinity can be made using suitable portable instruments (e.g. YSI model 85 T/S/O probe or “Seabird” CTD). Alternatively, temperature at the water surface can be measured using a hand-held (e.g. mercury) thermometer, at each location where juvenile salmon are caught. To determine water salinity, a water sample can also be collected from the sea surface into a suitable bottle at the same time and location. These water samples can subsequently be analyzed in the laboratory using a suitable precision instrument such as a Portasal<sup>®</sup> salinometer, which has an accuracy certified by the manufacturer of at least + or – 0.003 parts per thousand. Any instrument used to analyze water samples to determine salinity should be standardized before and after each set of water samples, using standard, reference quality saline solution commercially manufactured for this purpose, to verify the stability of the machine. Three flushes and a minimum of two readings should typically be taken for each water sample.

## C.4 Field data processing

In the field the catch from each fishing operation should immediately be recorded either manually or electronically. Manual recording of fish catch and sampling information may be facilitated by using water- proof paper that is pre-printed with a data template that includes blanks for the set location, time, date, and catches for the common fish species.

The fish catch and sampling data should be carefully checked to verify the accuracy and quality of the data. For example, the data for each fishing operation should be checked to ensure the time and date that were recorded are correct, and the specific locations (e.g. GPS coordinates) that were recorded in the field are actually within the boundaries of the study area and closely match the actual coordinates and name of the actual sampling locations. Verification of field data is important as a variety of data errors commonly occur and if not corrected these errors can result in incorrect or misleading conclusions. In many cases these are simply human errors made in recording or transcribing data under adverse conditions in the field.

When capturing juvenile wild salmon, particular attention should be given to correct identification of the fish species. For example, juvenile chum salmon are easily distinguished from juvenile pink salmon soon after these species enter the marine environment (e.g. by the highly visible “parr” marks (dark coloured vertical bands) on chum, and subsequently by the smaller size of scales in pinks). As the fish grow older and larger, however, pink and chum becomes increasingly difficult to distinguish by using only the external characteristics. Eventually additional internal features, such as the number and appearance of the gill rakers, must frequently be examined to confirm the species identifications (see Pollard et al. 1997).

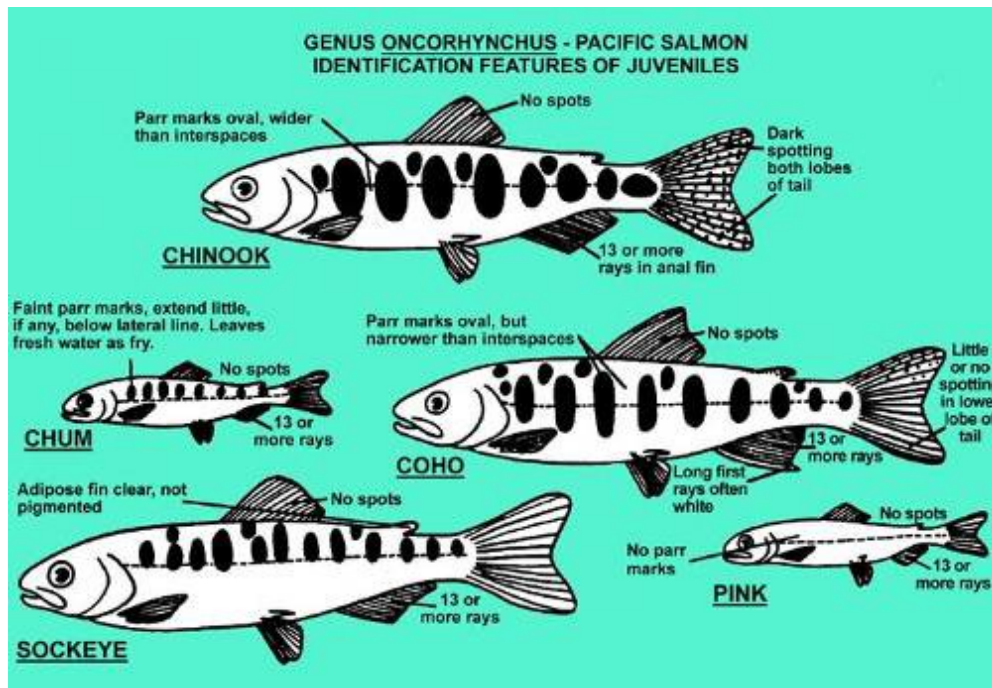


Figure 2. Pacific salmon feature [identification](#).

Capturing each fish alive into an individual plastic bag means that these internal characteristics cannot be examined without removing the fish from the bag and handling them. In studies of sea lice infections this may not be desirable because of the risk of losing sea lice from either the fish or the sample bag. Therefore, in the DFO purse and beach seine studies conducted in the Broughton from 2003–2005, the species identifications in the field were done as accurately as possible with the fish remaining inside the plastic bags. It was recognized that this would result in some errors in species identification, and therefore also some errors in the original field catch data. To correct



---

for these errors the final catch data were subsequently adjusted, based on the species identifications that were later confirmed in the subsequent analyses of the frozen fish samples back in laboratory. For example, assume that the original field catch data indicated that 200 chum and 200 pink were caught in a particular beach or purse seine set, and that 30 fish of each species were bagged and frozen. If the subsequent analyses of these frozen samples back in the laboratory indicated that 15 of these “pinks” were actually chum, then the original catch data were adjusted proportionally to 300 chum and 100 pinks captured.

## **D. CALIGID ZOOPLANKTON SAMPLING**

### **D.1 Background on motile stages of Caligids**

It is not clearly understood how the motile nauplii stages and copepodites of *Lepeophtheirus salmonis* and *Caligus clemensi* move or are transported in the inlets to reach the areas where salmonid smolts congregate on their outgoing migration to the sea. Caligid copepods are free living for a brief period of their life cycle, approximately 2 days for each nauplii stage and up to a week for the copepodid, depending on temperature and salinity (for example see Figure 2 in P&G 1). The developmental rates for free swimming stages are temperature dependent so they may be in the water column for as little as 5 days or as long as 15 days (Devine 2002). Generation time from hatch to adult, for *Lepeophtheirus salmonis*, can be 6 weeks at 9°C; higher temperatures decrease generation time and increase abundance (Hogans and Trudeau 1989). Making the correct identification of the planktonic stages can also be difficult (Johnson and Albright 1991).

### **D.2 Sampling design considerations**

Copepodite spread, horizontally and vertically throughout the water column, is not uniform which can make sampling a challenge. The following information from previous studies can help to determine sampling design temporally and geographically:

- Large numbers of free-swimming larval stages have been found in bays with and without farms peaking in the spring with a smaller peak in the fall (Costelloe et al. 1998).
- The copepodites show a distinct reverse diel migration to most planktonic organisms; moving to the surface during the day and into the deeper waters at night (Heuch et al. 1994).
- The nauplii move away from and the copepodites moves towards a light source (Novlaes-Flamairque 2000).
- Several experiments show that the copepodites sink to lower depths in the water column when entering low salinity waters and swim upwards in response to higher salinity (Heuch 1995).
- High numbers of copepodites have recently been shown to occur on the salt-water side of the intertidal shear zone between fresh and salt water close to river mouths (McKibben and Hay 2004).
- Periods of slack tide, mainly the high slack, has the highest density of copepodites occurring in the surface waters (Costelloe 1998).
- Hogans, in a report to DFO St. Andrews 1997, found the zooplankton samples with the greatest abundance of larvae occurred from inside fish pens and during peak yearly water temperatures. Costelloe (1998) showed that the #/m<sup>3</sup> of copepodites decreased with distance from pens.
- Off the bottom water sampling and suction samples of the top layer of sediment yielded poor results (one copepodite in 16 samples) (Costelloe et al 1998)

---

### D.3 Zooplankton Sampling Methodology

#### Vertical Net Haul

This method employs the following materials:

- a 200 to 250 $\mu$  mesh funnel net (SCOR, NorPac, Ring) with a 0.56m mouth opening
- a BONGO net can be used if it is beneficial to have duplicate samples
- a TSK flowmeter (or equivalent with one way rotation) offset in mouth
- a detachable cod end that should be approximately  $\frac{1}{2}$  to 1l capacity with appropriate mesh

A minimum 5kg weight is necessary to stabilize the net while sampling nearshore shallow sites. The deeper the tow the more weight is necessary. For a vertical net haul, the net is lowered into the water at 0.5m/sec to the desired depth and retrieved at 1 to 1.5m/sec. Sampling is done on the lee side of the vessel which maintains position and holds the wire angle to vertical. More wire is let out to compensate for wire angles greater than 5°. It is also useful to have an inclinometer to read wire angles. Smaller mouth openings are available for SCOR or NorPac. It is preferable to use the larger net sizes to be able to sample more volume for each unit of effort.



NorPac (ring net)

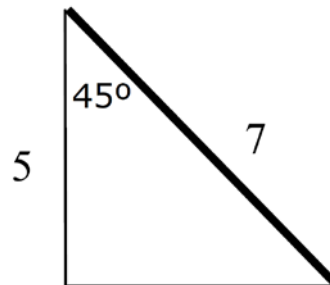


Bongo net

Figure 3. Zooplankton vertical net haul types.

#### Horizontal Net Haul

A table of desired depth versus wire angle should be made up ahead of time (45° wire angle requires 7m of wire out to achieve a 5m depth). This method employs the same nets as mentioned above but the weight needs to be shifted from the cod end to the mouth opening. A weighted planer is ideal. The net is lowered into the water as the boat moves up to speed of 2knts and enough wire is let out to reach desired depth. The duration of tow should be around 3 to 5 minutes at each selected depth. A net is pressure/temperature data logger can be attached to the net to record actual depth achieved. If possible, the line should be marked in 1m, 5m and 10m intervals to aid in achieving the correct depth.



For sampling in 1m to 3m water depth a smaller net diameter would be more appropriate. Towing needs to be at a steady pace and around 1m/sec to counteract net avoidance by zooplankton; a Zodiac is best for this work. A frame harness is used to suspend the net at the surface of the water; net is deployed on the nearshore side ahead of the wake as the boat is moved slowly through the shallows. This frame can also be hand deployed. As the person wades or walks the beach at a steady pace the net is deployed slightly ahead or to one side to avoid any disturbance or silting. Volume is calculated by distance traveled times the area of the net mouth times an efficiency factor for the net when a flowmeter is not being used.

Efficiency factors for various nets are calculated between .85 to .9 dependent on type of net and mesh being used.

There are multi-net samplers (MOCNESS, Bioness, VMPS) which can collect samples at discrete depths; i.e. 0-5m, 5-10m, 10-15m, etc. on a horizontal tow but they require a large sampling platform to operate due to size, electronics and weight of the sampling device. Neuston net is another sampling device that can be deployed behind a zodiac for horizontal surface tows but the length of the net and floatation apparatus makes for difficult handling and it is best deployed off a larger vessel.

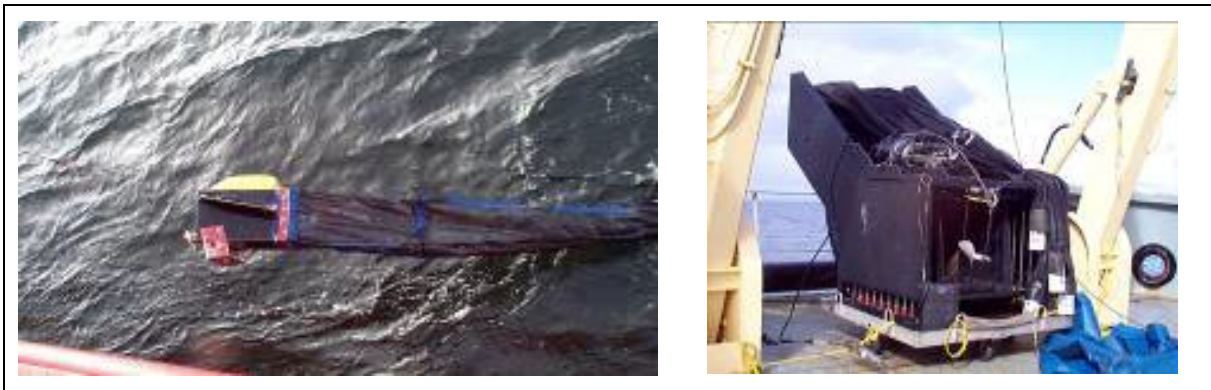


Figure 4. Horizontal net haul apparatus (left Neuston, right Bioness).

### Net mesh size

The nauplii and copepodites are greater than 0.4mm in length and around 0.2mm in diameter so a 200 $\mu$  to 250 $\mu$  mesh should be able to catch and retain animals of this size (see Table 3). Going to a finer mesh will capture more phytoplankton and lead to clogging of the net. A 150 $\mu$  mesh can be used if the phytoplankton load is not heavy but attention to blockage on the net is important to assure that water is moving through the mesh. Finer mesh also leads to a pressure wave building in the mouth opening as the water is unable to move through quickly. This needs to be gauged and rate of towing adjusted. Black nylon mesh makes the net less visible in the water but for copepods white is just as effective as their escape response is limited.

Table 3. Average Sizes of Lice.

Species stage	Length (mm)	Diameter (mm)
<i>Lepeophtheirus salmonis</i> nauplii I and II	0.5-0.56	0.2-0.22
<i>Lepeophtheirus salmonis</i> copepodid	0.7	0.28
<i>Caligus clemensi</i> nauplii I and II	0.46-0.53	0.2-0.22
<i>Caligus clemensi</i> copepodid	0.66	0.25



### Environmental Variable Measurement

With each plankton tow, corresponding environmental variables of the water column vertical profiles of salinity and temperature should be measured. A Seabird model SBE 19 Seacat is ideal for this work. It has internal data logging capabilities and will hold up to 50 casts of 100m or less depth in memory. In lieu of an electronic recording device water measurements can be done as per section C.2.

Figure 5. Environmental measurement device.

## D.4 Sampling design considerations

There are several items to consider when designing a sampling program targeting the free-living planktonic stages of sea-lice. If the purpose is to find out when sea-lice larvae are most 'catchable' in BC waters then previous work in Europe (referenced above) suggests that three 'environmental' cycles affect this.

### 1. Temporal cycles

- diurnal or diel rhythms
  - **Sampling suggestion:** For 2-3 sampling days in sequence duplicate ~ 5 minute tows at hourly intervals during daylight hours and repeat for night cycle.
- yearly cycles, particularly spring vs. fall

### 2. Stage of tide (flood to HW slack to ebb to low water slack)

- **Sampling suggestion:** expect highest catch during daylight so duplicate tows at ~hourly intervals through a HLH or a LHL sequence during daylight on two successive days, ideally at 2 different but nearby sites. This is simpler if done when highs and lows are near equal (neap tide).

### 3. Phase of tide (spring vs. neap).

- As above but sampling over a tidal cycle, on the spring and neap tides

In inlets with fish farms, a sampling site should be chosen in an area of active net pens, but not so close that current swirls and harvesting/treatment practice at a single pen dominate the supply of larvae.

### Some Logistics Sampling Caligid Planktonic Stages

Some logistic issues in sampling design result from the known (and unknown) characteristics of *Lepeophtheirus salmonis*:

1. Much of the total larval development occurs within the eggs, while they are still attached as egg strings to ovigerous females on fish. There is some evidence that hatch timing is episodic at fortnightly to seasonal time scale. (Boxshall and Defaye ed 1993)
2. Duration of the three free-living planktonic stages is short (roughly 5 days at Broughton ambient temperatures). Expected peak catch rates are typically low: probably 2-3 orders of magnitude rarer than the non-parasitic copepods that will be caught in the same samples. The sorting species problem is solvable but time consuming.

- 
3. Once separated from the rest of the zooplankton, identification to species will be difficult for 2 of the 3 planktonic stages of the parasitic caligids—the nauplii 1 and 2 which are quite small, similar in body shape, colour and armature. Live nauplii have pigment patterns which can be used in an aid to identification. The copepodite stage is larger with more legs and appendages allowing additional taxonomic cues to assist in proper identification
  4. More serious is that European results show strong (roughly 10-fold) variation in apparent abundance at any single site. Several time scales interact: day vs. night, tidal stage (high vs. low), and perhaps tidal phase (spring vs. neap). Much of this variation is almost certainly behaviour-mediated variation in catchability, rather than real variation in population size. Although the European experience can give us some reasonable measures of what to expect, because of differences in oceanography, we do not know if European protocols for sample timing (daylight, near high tide) would maximize BC catch.
  5. There is also European evidence for strong spatial patchiness of the larvae, and in particular, accumulation in shallow near-shore environments. Again, we do not know the degree to which European results apply in BC fjords.

#### Definitions

- **Diel** means occurring on a daily basis in the sense of a 24-hour period rather than the time between sunrise and sunset. Thus a "diel variation" is one that occurs regularly once per 24-hour period.
- **Diurnal**: Daily as in belonging to the daytime; active by day; opposite of "nocturnal".
- **Nocturnal**: Active during the night; opposite of "diurnal".
- **Neap tide**: The lowest level of high tide that occurs when the difference between high and low tide is least. Neap tide happens twice a month, in the first and third quarters of the moon.

#### D.5. Preservation of zooplankton samples

The net should be raised out of the water and rinsed with the same water as sampled; spraying from the outside of the net, starting at the mouth and working all material down the funnel to the cod end. Large changes in salinity can cause rupture of membranes in zooplankton. If the water is thick with phytoplankton, it may be necessary to wash the cod end onto a larger sieve (made of the same mesh as the net). More surface area available makes it easier to reduce the sample down to a manageable size. If there is considerable debris in the water then a series of stacked sieves can be used to remove larger chunks, usually a 4mm over a 2 mm on a 0.2 mm. For formalin preserved samples, allow 1 part sample to 2 parts preservative at a minimum; optimum is 1 part sample to 4 parts preservative. Formalin should be made up ahead of time: 10% formaldehyde in filtered sea water buffered with borax. Unbuffered formalin will destroy calcium structures (molluscs) and break down hydrostatic skeletons (Medusae, Chaetognaths) over time. For frozen samples, remove as much water as possible and freeze quickly. An alcohol bath freezer is the best means to preserve a sample; samples are frozen in less than 5 minutes at -32°C. Dry ice is the next best method followed by ice chips in a cooler; block ice or freezer packs being last choice for freezing zooplankton samples.

[The world of Copepods](#) is a good reference for the preservation and examination of zooplankton, put together by Janet Reid for the Smithsonian Institution

#### E. SAMPLING ADULT SALMON: ESCAPEMENT ESTIMATION

All salmon stock management systems rely on estimates of annual and seasonal variations in total abundance determined at each of several life history stages by a variety of different methodologies. Because of the historic focus on adult salmon as a harvestable resource, the most basic fisheries

---

management system depends on annual assessments of total returns of adults. Total returns (production) are comprised of all fish of a given species accounted for in fishing mortality (catch plus incidental mortality) plus the remainder that escape fisheries to return as spawners in their natal freshwater streams. Consequently, the number of spawners that succeed in returning to a given lake or stream is commonly known as the “escapement”. Thus, annual assessments of salmon escapement are critical activities required to satisfy federal and provincial fisheries agency mandates in several areas including:

- 1) stock conservation,
- 2) compliance with fiduciary obligations to aboriginal fisheries groups,
- 3) harvest management,
- 4) habitat conservation and management,
- 5) indexing and maintenance of ecosystem integrity, and
- 6) stock and habitat research.

It is important to recognize, however, that the fishing mortality on individual salmon populations (i.e., returns by species from a spawning stream) are very seldom known due to the extensive mixing of salmon populations in fisheries and the numerous fisheries that typically impact fish from one population. Catch near a spawning stream may be referred to as the terminal catch (and assumed to be only of local stream origin) but such fisheries are usually only a portion (and usually small) of the total fishing impact. Consequently, extensive efforts are directed to monitoring the numbers of salmon “escaping” fisheries and returning to their natal streams to reproduce (spawn).

The collection of salmon escapement information involves a set of activities including specification of stream survey enumeration plans, training of field surveyors, data gathering and documentation, data review and processing, data analysis or synthesis of summary estimates, and release of the data to both internal (i.e. fisheries agency personnel) and external clients. The coordination of these activities is performed by federal (Stock Assessment Division of Fisheries and Ocean’s Science Branch) or provincial stock assessment groups.

## **E.1 Standards**

Standards are intended to provide information of a specific quality, in terms of accuracy, precision, and reliability, to attain specific objectives. The standards for salmon escapement programs vary depending on scientific objectives. Ultimately the choice of standards rests with the principal investigator and balance between program objectives, resources, and characteristics of the species and environment.

The accuracy, precision and reliability of escapement estimates are affected by characteristics such as the assessment method, species behaviour, hydrologic and other environmental conditions. It is often highly demanding on resources to gain information on the accuracy of a specific assessment method under certain conditions, so such studies are typically rare. However, there are several types of assessment methods with common aspects of accuracy, precision, and reliability that are applied (Table 4). In general, the Department’s strategy in collating escapement data over many years has been to provide a consistent index of numbers of spawners (i.e., a consistent measure of change in annual returns). Highly accurate and precision data needed for estimation of productivity and studies of population dynamic processes have only been maintained on “indicator” populations due to the demanding nature of these programs. The Department has evolved to this compromise in information due to the thousands of Pacific salmon spawning streams to monitor versus the fiscal and personnel resources available to collect these data annually.

---

## E.2 Protocols and guidelines

Escapement assessment methods are well described in the fisheries literature, including peer-reviewed papers, published manuscript reports, and text books. Study designs vary among species, locations, and environmental conditions of the streams. To achieve a balance between objectives, resources, species behaviour and environmental conditions, a wide diversity of methods have evolved to estimate salmon spawner numbers. Typically assessment methods are described in manuscript reports, stream narratives, or stock assessment reports to communicate the quality of the annual escapement estimate or assessment program.

General guidelines for developing a spawning escapement program are of little to no value without considering the environmental conditions involved, the experience of the personnel, the objective of the monitoring program, and logistical limitations (number of staff, financial resources, safety considerations, etc). However, to assist in the development of such programs, Dr. Kim Hyatt has provided a bibliography of survey methods used in salmon escapement enumeration (Chapter 2, Addendum 2). Our best advice for those interested in developing a monitoring program for salmon spawning escapements is to consult with an experienced field biologist, clearly define the objectives of the program (levels of accuracy and precision necessary), and realistically assess the environmental conditions expected in the streams. While substantial technical literature exists on escapement sampling, local knowledge of the streams and salmon behaviour will be useful in designing surveys.

## E.3 Escapement Estimation Methodology for Pink salmon (an example)

### Collection of Field Information

- All escapement estimates for Mainland pink stocks are derived through visual counts (via aerial, stream walk or snorkel surveys)
- Each system is divided into standard survey sections that are easily identified and repeatable over time.
- In order to ensure appropriate coverage of the return timing and abundance, the escapement plan requires multiple surveys (usually 5 -6) at a frequency of every 10-14 days over the duration of the return run-timing.

**Visual-Based Estimates** (example for aerial surveys but similar methodology is used for swim and stream walk surveys):

- Section counts of pink salmon are conducted independently by 2 observers within the same helicopter (no discussion of numbers occurs during the flight of a particular system)
- The observer counts by section are then averaged to provide the number of fish observed for that section. These survey section counts are then expanded by an agreed 'observer efficiency' based on the countability of the fish in the system. This is currently a subjective expansion based on the experience of the observers. Further work is required to calibrate the observer efficiency of these flights.
- Each section estimate is then summed for the entire system and then expanded based on the percent of the population covered. In most cases where the system is flown the % population is 100% (i.e.100% of the habitat utilized by this species was covered by the flight). In some instances this will change based on the amount of coverage accomplished on a given assessment flight (i.e. Weather problems forcing only partial coverage of the habitat).
- This information is then transferred to the regional escapement database where further analysis is conducted.

---

### **Compilation of Escapement Data for Analyses:**

Once the escapement collection program has been completed and the information of each assessment event is compiled, the process of estimating the total return to the river is initiated. The Stream Escapement Narrative (SEN) which houses the final escapement estimate for a specific species, system and year is collated. How the estimates of escapement are derived depends on the level of escapement coverage during the in-river migration timing of that species. In order to facilitate the process of determining which systems and species require more detailed analysis a function has been created to assess this (Figure 6 below). The increased visitation frequency attributed to the directed escapement focus for Mainland Pink systems typically results in further detailed analysis of the escapement data.



Table 4. Characterization of escapement estimates based on associations between survey method, reliability, accuracy and precision.

Estimate Type	Survey Method(s)	Analytical Method(s)	Reliability (within stock comparisons)	Units	Accuracy	Precision	Documentation <sup>1</sup>
Type-1, True Abundance, high quality	total, seasonal counts through fence or fishway; virtually no bypass	simple, often single step	reliable resolution of between year differences >5% (in absolute units)	absolute abundance	actual, very high	infinite i.e.+ or - zero%	detailed SIL(s), SEN, field notes or diaries, published report on methods
Type-2, True Abundance, high quality	high effort (8 or more trips), standard methods (e.g. mark-recapture, serial counts for area under curve with direct survey life, etc...)	simple to complex multi-step, but always rigorous	reliable resolution of between year differences >10% (in absolute units)	absolute abundance	actual or assigned estimate and high	actual estimate, high to moderate	detailed SIL(s), SEN, field notes or diaries, published report on methods
Type-3, True Abundance, medium quality	low effort (2-3 trips) of a standard method (e.g. peak count method) expanded by a factor to estimate true abundance	simple multi-step index surveys, but rigorously calibrated to estimates of true abundance (Type-1 or Type-2)	reliable resolution of between year differences >25% (in absolute units)	absolute abundance	actual or assigned estimate and moderate	actual estimate, moderate	detailed SIL(s), SEN, field notes or diaries, published report on methods examining bias and precision of applied factors in retrospect
Type-4, Relative Abundance, medium quality	high effort (5 or more trips), standard methods (e.g. equal effort surveys executed by walk, swim, overflight, indirect survey life etc.)	simple to complex multi-step, but always rigorous	reliable resolution of between year differences >25% (in absolute units)	relative abundance linked to method	assigned range and medium to high	assigned estimate, medium to high	detailed SIL(s), SEN, field notes or diaries, published report on methods
Type-5, Relative Abundance, low quality	low to moderate effort (1-4 trips), known survey method but no expansion factor	simple analysis by known methods, not calibrated	reliable resolution of between year differences >200% (in relative units)	relative abundance linked to method	unknown assumed fairly constant	unknown assumed fairly constant	complete SEN or equivalent with sufficient detail to verify both survey and analytical procedures
Type-6, Relative Abundance, poor quality	low effort (e.g. 1 trip), use of vaguely defined, inconsistent or poorly executed methods	unknown to poorly defined; inconsistent or poorly executed	uncertain numeric comparisons, but high reliability for presence or absence	relative abundance, but vague or no i.d. on method	unknown assumed highly variable	unknown assumed highly variable	incomplete SEN, only reliable to confirm estimate is from an actual survey
Type-7, Presence or Absence	any of above	not required	moderate to high reliability for presence or absence	(+) or (-)	medium to high	unknown	any of above sufficient to confirm survey and reliable species i.d.

1. SIL is Stream Inspection Log and SEN is Salmon Escapement Number.

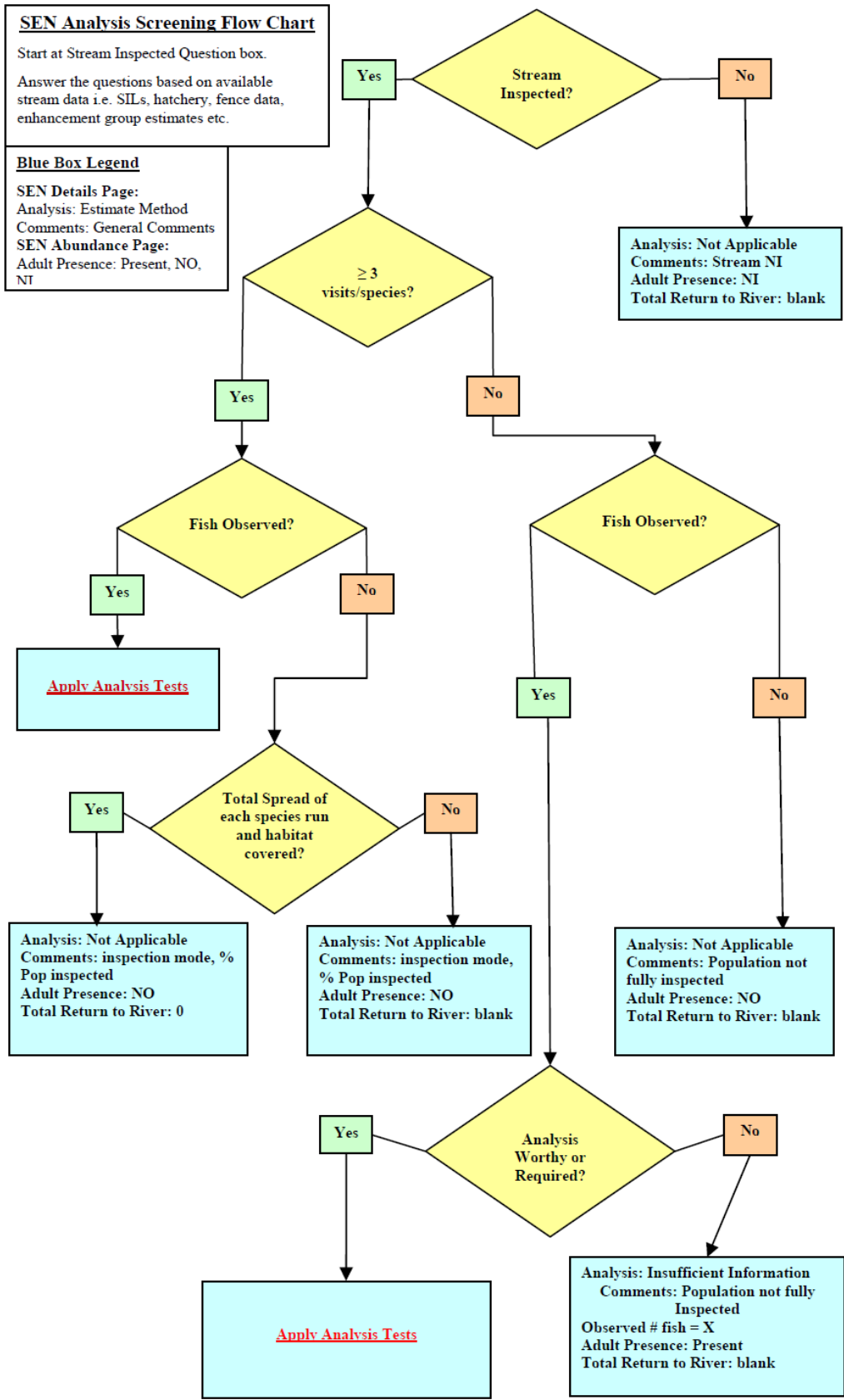


Figure 6. Analysis Flowchart.

## Estimation of total numbers of spawners (escapement)

Two main estimation methods are used to finalize escapement estimates:

1. Peak Live + Dead, and
2. Area Under the Curve (AUC, see Addendum 2).

The first, Peak Live + Dead, usually involves 3-4 reasonably spaced observations (surveys) centered on the historical peak return timing of that species within a system. The peak live estimate is added to the dead observed on that peak day to provide an estimate of total return to river. Due to the apparent long residence time of pinks in many of systems, this simple estimator can be considered as reasonable index of total annual abundance or escapement. This has typically been the main methodology employed throughout the historic time series of escapement data for many of the mainland inlet pink salmon steam systems (Figure 7).

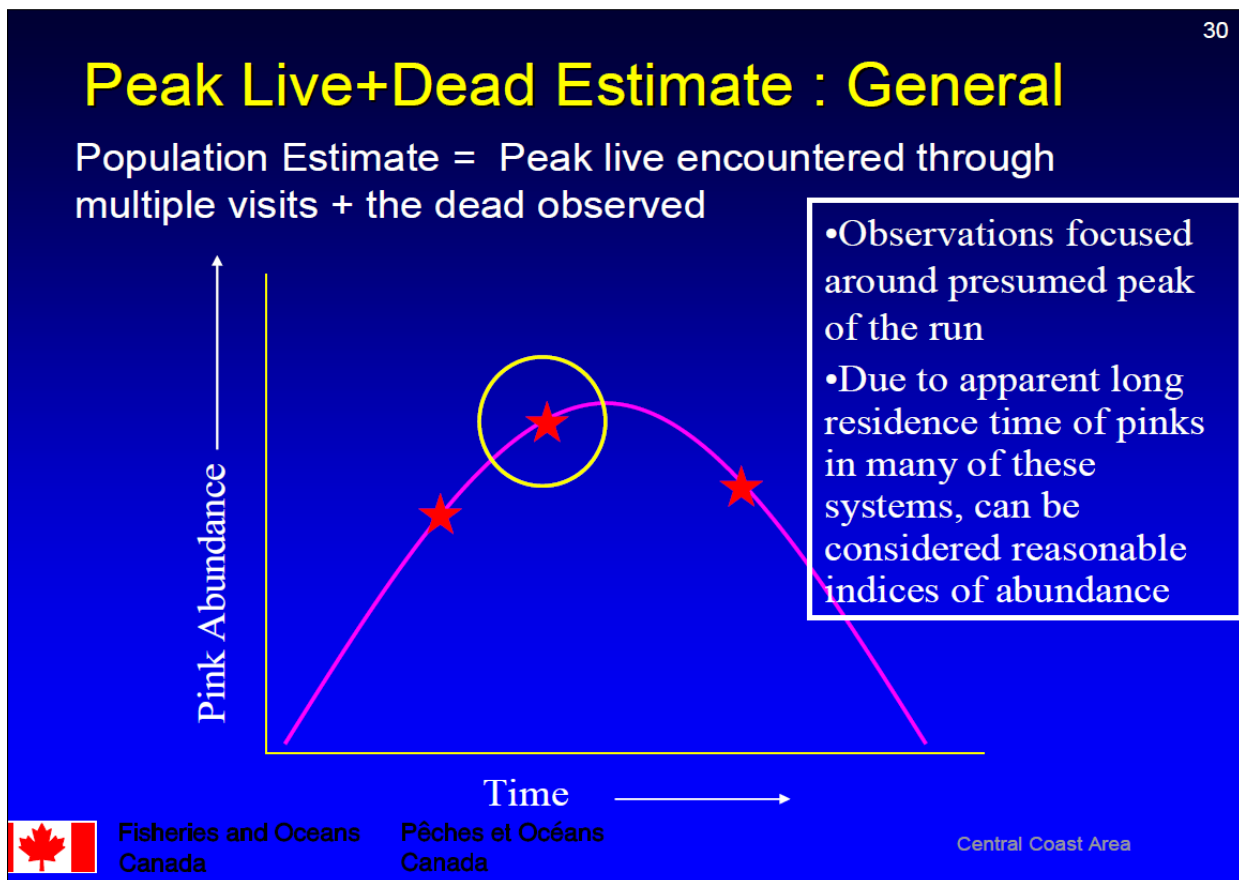


Figure 7. Graphical representation of Peak Live + Dead Escapement estimate.

The second method, AUC, requires a greater frequency of surveys and a better understanding of the residence time of that species. Currently systems that have reasonable coverage (5-6 visits appropriately spaced) are candidates for AUC analysis. A spawner curve is created based on each daily estimate of escapement and the area under that curve is estimated (via trapezoidal or other algebraic means). The estimated area under the curve, or total fish days, is then divided by the stream residence time of that stock to provide the estimate of escapement for the species

## Area Under the Curve Estimation: General

Population Estimate =  
 $AUC/SL$

AUC = Area under the Escapement curve  
 or total fish days

SL = Survey life, or the number of days the  
 average fish is within the survey area

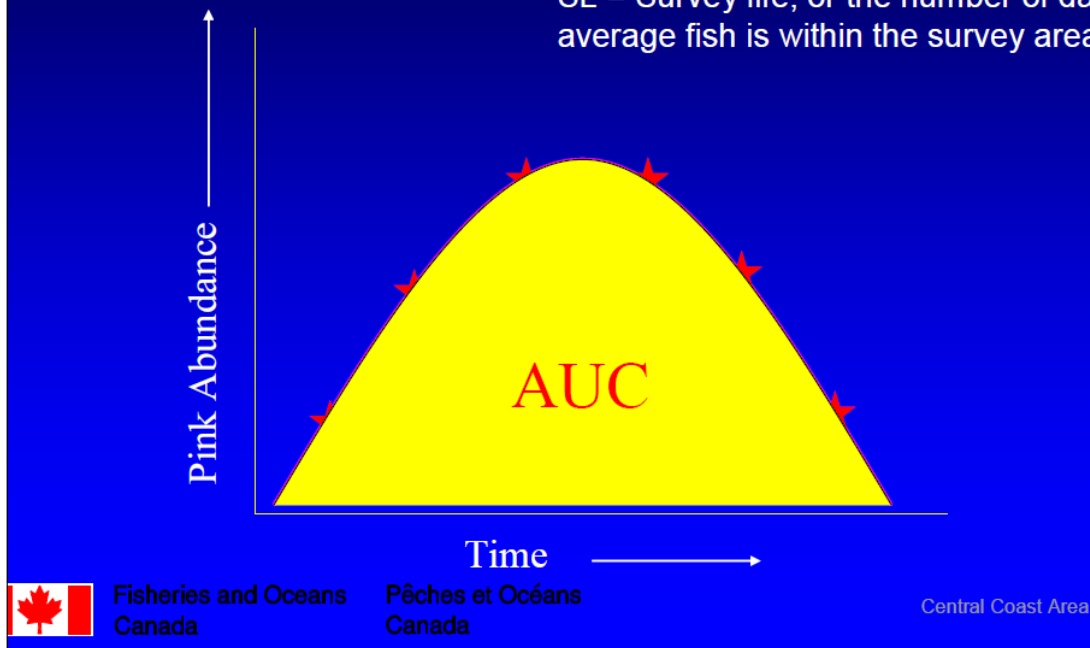


Figure 8. Graphical representation of AUC Escapement estimate.

The estimates of stream residence time is key to an accurate estimate of the total escapement and will vary depending on the system, environmental conditions and the quality of the information provided by the escapement observations. In instances where there is little information on the specific system, more generalized estimate of pink salmon residence time are utilized (e.g., see Perrin and Irvine 1990, Irvine et al. 1993, Irvine and Nelson 1995). As there are no direct measurements of survey life for these mainland pink systems, other indicators of survey life are utilized. One option is looking at equivalence points between the cumulative live and dead estimates for the return. This requires coverage throughout the spawning and die-off period of the return. The duration of days between the 10, 50 and 90% point on the two cumulative periods are averaged to provide the estimate of residence time (Figure 9).

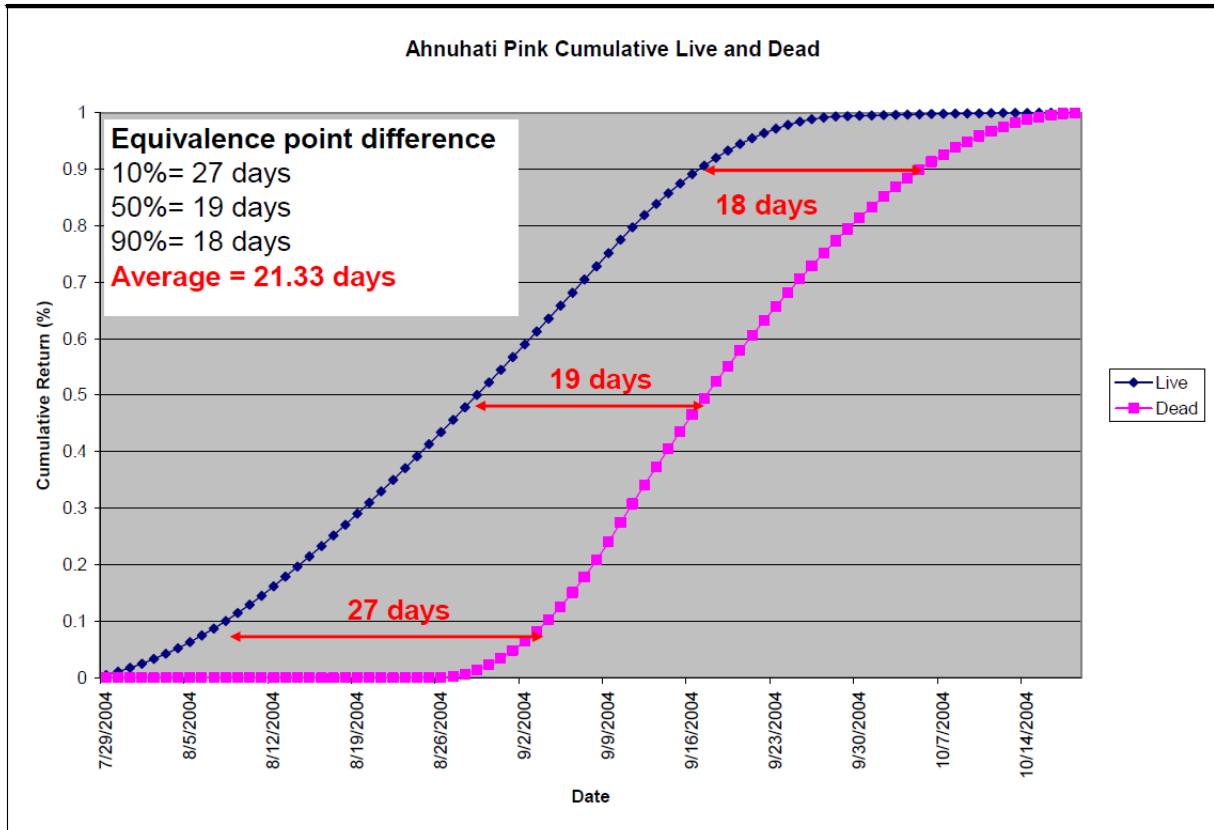


Figure 9. Graphical representation of survey life estimates by averaging equivalence point difference between cumulative live and dead.

Both estimation methods described above involve significant levels of uncertainty due to the use of visual counts and estimates of stream residence time. The Department is currently in the process of incorporating this uncertainty through various simulation models to provide the probability distribution of the escapement estimates.

## F. REFERENCES

### References for Fish Sampling:

- Beamish, R.J., D. McCaughran, J.R. King, R.M. Sweeting, and G.A. McFarlane. 2000. Estimating the abundance of juvenile Coho salmon in the Strait of Georgia by means of surface trawls. *N. Amer. J. Fish. Manage.* 20: 369-375.
- Beamish, R.J., C.M. Neville, R.M. Sweeting and N. Ambers. 2005. Sea lice on adult Pacific salmon in the coastal waters of Central British Columbia, Canada. *Fish. Res.* 76:198-208.
- Carpenter, S.R. and P.A. Matson. 1990. Special feature: Statistical analysis of ecological response to large-scale perturbations. *Ecology* 71(6): 2037-2068.
- Doubleday, W.G. and D. Rivard (editors). 1981. Bottom trawl surveys. *Can. Spec. Publ. Fish. Aquat. Sci.* 58.
- Eberhardt, L.L. and J.M. Thomas. 1991. Designing environmental field studies. *Ecol. Mono.* 61(1): 53-73.
- Green, R.H. 1979. *Sampling Design and Statistical Methods for Environmental Monitoring*. John Wiley & Sons, New York.

- 
- Hartt, A.C. 1966. Migrations of salmon in the North Pacific Ocean and Bering Sea as determined by seining and tagging, 1959-1960. INPFC Bull. 19.
- Heffner, R.A., M.J. Butler, and C.K. Reilly. 1996. Pseudoreplication revisited. *Ecology* 77(8): 2558-2562.
- Hurlbert, S.H. 1984. Pseudoreplication and the design of ecological field experiments. *Ecol. Mono.* 54: 187-211.
- Hurlbert, S.H. 2004. On misinterpretation of pseudoreplication and related matters: a reply to Oksanen. *Oikos* 104(3): 591-597.
- Holst, J. C. and A. McDonald. 2000. FISH-LIFT: a device for sampling live fish with trawls. *Fish. Res.* 48: 87-91.
- Irvine, J. R. and T. C. Nelson (Editors). 1995. Proceedings of the 1994 salmon escapement workshop plus an annotated bibliography on escapement estimation. *Can. Manuscr. Rep. Fish. Aquat. Sci.* 2305: 97 p.
- Irvine, J. R., J. F. T. Morris, and L. M. Cobb. 1993. Area-under-the-curve salmon escapement estimation manual. *Can. Tech. Rep. Fish. Aquat. Sci.* 1932: 84 p.
- Johnson, S.C., and L. J. Albright,. 1991. The developmental stages of *Lepeophtheirus salmonis* (Krøyer 1837) (Copepoda: Caligidae). *Can. J. Zool.* 69, 929-950.
- Kabata, Z. 1972. Developmental stages of *Caligus clemensi* (Copepoda: Caligidae). *J. Fish. Res. Bd. Can.* 29, 1571-1593.
- Kabata, Z. 1973. The species of *Lepeophtheirus* (Copepoda: Caligidae) from fishes of British Columbia. *J. Fish. Res. Bd. Can.* 30, 729-759.
- Krebs, C.J. 1999. *Ecological Methodology*, 2<sup>nd</sup> Ed. Benjamin-Cummings Publ. Menlo Park, CA.
- Krkosek, M., A. Morton and J.P. Volpe. 2005. Nonlethal assessment of juvenile pink and chum salmon for parasitic sea lice infections and fish health. *Trans. Am. Fish. Soc.* 134:711-716.
- Margolis, L., G.W. Esch, J.C. Homes, A.M. Kuris, and G.A. Schad. 1982. The use of ecological terms in parasitology (report of an ad hoc Committee of the American Society of Parasitologists). *J. Parasitol.* 68, 131-133.
- Michener, W.K. 1997. Quantitatively evaluating restoration experiments: Research design, statistical analysis, and data management considerations. *Restoration Ecology* 5(4): 324-337.
- Morton, A. and R. Williams. 2003. First report of the sea louse, *Lepeophtheirus salmonis*, infestation on juvenile pink salmon *Oncorhynchus gorbuscha*, in nearshore habitat. *Can. Field-Nat.* 118: 634-641.
- Morton, A., R. Routledge, C. Peet, and A. Ladwig. 2004. Sea Lice (*Lepeophtheirus salmonis*) infection rates on juvenile pink (*Oncorhynchus gorbuscha*) and chum (*Oncorhynchus keta*) salmon in the nearshore marine environment of British Columbia, Canada. *Can. J. Fish. Aquat. Sci.* 61: 147-157.
- Neter, J., M. H. Kunter, C. J. Nactsheim and W. Wasserman (1996). *Applied Linear Statistical Models*. 4th Edition. Richard D. Irwin, Chicago, Ill.
- Oksanen, L. 2001. Logic of experiments in ecology: is pseudoreplication a pseudoissue? *Oikos* 94(1): 27- 38.
- Oksanen, L. 2004. The devil lies in details: reply to Stuart Hurlbert. *Oikos* 104(3): 598-605.
- Pearcy, W.G. and J.P. Fisher. 1990. Distribution and abundance of juvenile salmonids off Oregon and Washington, 1981-1985. NOAA Tech Rep 93.

- 
- Perrin, C. J., and J. R. Irvine. 1990. A review of survey life estimates as they apply to the area-under-the-curve method for estimating spawning escapement of Pacific salmon. Can. Tech. Rep. Fish. Aquat. Sci. 1733: 49 p.
- Pollard, W.R., G.F. Hartman, C. Groot, P. Edgell. 1997. Field identification of coastal juvenile salmonids. Harbour Publ., Madeira Park, B.C. (ISBN 1550171674)
- Quinn, G.P. and M.J. Keough. 2002. Experimental Design and Data Analysis for Biologists. Cambridge Univ. Press, Cambridge, U.K.
- Sokal, R.R. and F.J. Rohlf. 1995. Biometry, 3<sup>rd</sup> Ed. W.H. W.H. Freeman, San Francisco, CA..
- Tucker, C.S., C. Sommerville, and R. Wooten. 2000. The effect of temperature and salinity on the settlement and survival of copepodids of *Lepeophtheirus salmonis* (Kroyer, 1837) on Atlantic salmon *Salmo salar* L. J. Fish Diseases 23: 309.
- Walters, C.J. 1986. Adaptive Management of Renewable Resources. McGraw-Hill, New York, NY.
- Walters, C.J., J.S. Collie, and T. Webb. 1988. Experimental designs for estimating transient responses to management disturbances. Can. J. Fish. Aquat. Sci. 45:530-538.

### References for Caligid Zooplankton Sampling

- Boxshall G. and D. Defaye editors. 1993. Pathogens of Wild and Farmed Fish: Sea Lice. Ellis Horwood Limited.
- Costelloe, M. et.al. 1998. Planktonic distribution of sea lice larvae, *Lepeophtheirus salmonis*, in Killary harbour, west coast of Ireland. J. Mar. Biol. Assoc. UK 78:853-874.
- Costelloe, M., J. Costelloe, N. Coghlan, G. O'Donohoe, and B. O'Connor. 1998. Distribution of the larval stages of *Lepeophtheirus salmonis* in three bays on the west coast of Ireland. ICES J. Mar. Sci. 55:181- 187.
- Costelloe, M., J. Costelloe, and N. Roche. 1996. Planktonic dispersion of larval salmon lice, *Lepeophtheirus salmonis*, associated with cultured salmon, *Salmo salar*, in western Ireland. J. Mar. Biol. Assoc. UK 76:141-149.
- Devine, G. 2002. SEARCH: Sea Lice Biology
- Heuch, P.A. 1995. Experimental evidence for aggregation of salmon louse copepodids (*Lepeophtheirus salmonis*) in step salinity gradients. J. Mar. Biol. Ass. U.K. 75: 927-939.
- Heuch, P.A., A Parsons and K. Boxaspen. 1994. Diel vertical migration: A possible host-finding mechanism in salmon louse (*Lepeophtheirus salmonis*) copepodids? J. Fish. Aquat. Sci. 52: 681-680.
- Hogans, W. E. 1997. Planktonic density and dispersion of larval sea lice *Lepeophtheirus salmonis* (Copepoda:Caligida) and its relationship to infection dynamics and sea cage site location in the lower Bay of Fundy, New Brunswick. Report to DFO, St. Andrews, N.B. 17p.
- Hogans, W. E. and D. J. Trudeau. 1989. Preliminary studies on the biology of sea lice, *Caligus elongatus*, *Caligus curtusi* and *Lepeophtheirus salmonis* (Copepoda:Caligida) parasitic on cage-cultured salmonids in the lower Bay of Fundy. Can. Tech. Rep. Fish. Aquat. Sci. 1715: iv + 14p.
- Johnson, S.C. and L. J. Albright. 1991. The developmental stages of *Lepeophtheirus salmonis* (Kroyer 1837) (Copepoda:Caligidae). Can. J. Zool. 69: 929-950.
- McKibben, M. and Hay, D.W. 2004. Distributions of planktonic sea lice larvae *Lepeophtheirus salmonis* in the inter-tidal zone in Loch Torridon, Western Scotland in relation to salmon farm production cycles. Aqua. Res., 35, 742-750.

- 
- Novales-Flamarique, I. 2000. Visual responses of the parasitic sea louse. *J. Exp. Biol.* p. 1649-1657.
- Omori, M. and T. Ikeda, 1984. *Methods in Marine Zooplankton Ecology*. John Wiley & Sons, New York.
- Pahl, B.C., Cole, D.G. and Bayer, R.C. 1999. Sea Lice Control II: Evaluation of a photomechanical device as an alternative sea lice control strategy. *J. App. Aquac.* Vol. 9(3): 75-88.



---

## **P&G 2 ADDENDUM 1: TRAWL SURVEY DESIGN**

The trawl survey is used to estimate the number of juvenile salmon in the study area. This is a recognized method of estimating juvenile abundance and is used regularly to sample juvenile salmon in the Strait of Georgia (Beamish and Folkes 1998, Beamish et al. 2000). The methods and procedures presented here are specific to sampling juvenile salmon with trawl gear in Queen Charlotte Strait and inlets on its eastern boundary and in Rivers and Smith inlets. The level of sampling required was determined using the results of an initial survey conducted in 2003.

### **Stratification and Sampling**

#### **Stratification**

Stratification is a commonly used technique in trawl surveys. Several factors may contribute to the decision to stratify an area, including: geographic differences, administrative convenience, or differences in abundance based on past surveys. Stratification may also occur for different depths. If a heterogeneous population can be divided into homogenous subpopulations (strata), then stratification may produce a gain in precision in the estimates of the characteristics of the population (e.g., gain in precision in estimate of mean abundance). Precise estimates can be obtained for each stratum, and these estimates can then be combined into a precise estimate for the population (Cochran 1977).

The survey area is stratified into 6 strata based on both the 2003 survey data and geographic differences. These strata are: Rivers Inlet, Smith Inlet, Queen Charlotte Strait, Kingcome Inlet, Knight Inlet, and Tribune Inlet. By stratifying the area into 6 strata it is possible to attain abundance estimates for each individual stratum as well as an estimate of overall abundance. It should be noted that the Broughton strata (Kingcome, Knight and Tribune) are more geographically homogeneous than the other 3 strata and could possibly be grouped together into a single stratum. Also, Knight and Tribune Inlets are more similar with respect to haul density and abundance estimates (based on 2003 data), thus could also be grouped together.

#### **Stratified Systematic Sampling**

Random sampling is ideal in survey situations where the constraints of cost and time are negligible and the sample size is large (500+). However for this survey the logistics of a stratified simple random survey are not very convenient, thus a systematic survey with a set track-line will be used.

There are also benefits to using a systematic survey. For this survey, systematic sampling will spread sampling stations more evenly over the population. This is of particular importance when the sample size is small.

#### **Pre-set track-line**

A systematic design should have a pre-set track-line. For this survey, the track-line will always be on an angle; cutting diagonally back and forth across each stratum. This pattern should be used for both the inlets and for the larger open areas such as QC Strait. It is important that the ship *does not* haul in a parallel direction to the shore. This applies to both near-shore and off-shore hauls. This is important because if a density gradient exists moving out from the shoreline then sampling parallel to the shore (at any distance from shore) will bias the sample. Instead, hauling at an angle to the shore allows each sample to be taken *across* the density gradient (Figure 1).

#### **Homogeneous sampling intensity (a semi-randomized design)**

It is important to ensure homogeneous sampling intensity with respect to the shoreline. If done correctly, this survey can be referred to as a semi-randomized design (which is superior to a

strictly stratified design). When the sampling intensity is equal with respect to the shoreline it removes biases associated with sampling intensity. In this way each distance from the shore has an equal probability of being sampled. This will ensure this survey can stand up to the traditional critiques of a systematic design (i.e., ensures survey is not sampling a cyclic pattern).

Each haul will be 30-min at 5-knots. This is approximately 4.6-km dependent on tides, winds and currents. For a given stratum the shore-to-shore distance is calculated. In order to maintain homogeneous sampling intensity, the position of hauls will alternate along each leg of the track-line (see Figure 1). In this example the design results in 6 near-shore hauls and 6 off-shore hauls.

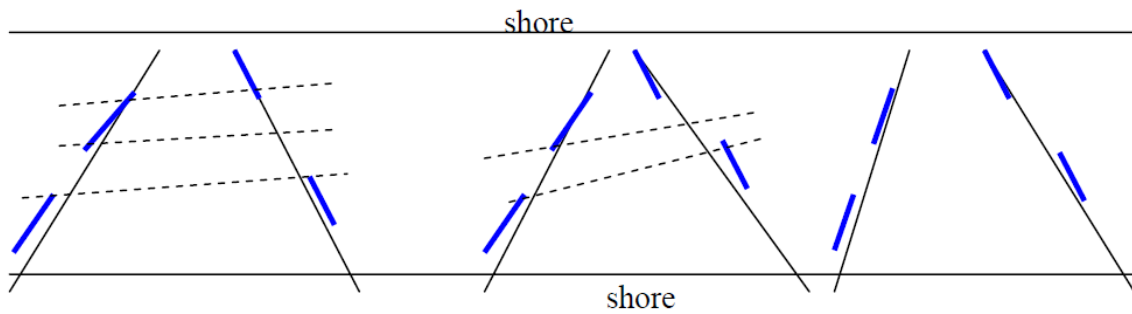


Figure 1. Diagrammatical example of shore-to-shore sampling haul positions to ensure homogeneous sampling intensity.

### Randomize distance between each leg of the track-line

The distance between each leg of the track-line should be random (see Figure 2). These distances will be chosen using a random number generator.

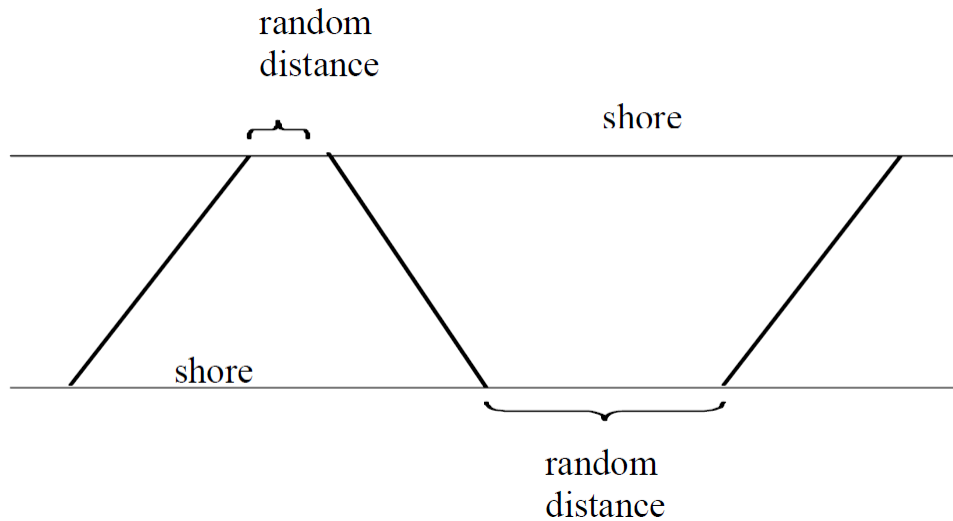


Figure 2. Diagrammatical example of the effect of using random distances for each track-line in shore-to-shore sampling.

Individual strata can be surveyed in any order.

### Haul Allocation

In this analysis, haul allocation to each stratum is calculated based on the 2003 trawl survey data. This is carried out using a Monte Carlo optimization model. This model determines the optimum

---

sampling pattern (over all strata) required to minimize the variances in the overall abundance estimates.

**Option 1**

In Option 1 hauls are allocated to each stratum based on the 2003 data by minimizing the variance in abundance estimate over *all strata combined*.

The following assumptions are made:

- Number of simulations – 10,000
- Maximum number hauls – 100
- Minimum number hauls in each stratum – 5

Table 1.

<b>Stratum</b>	<b>Minimum no. hauls</b>
Rivers Inlet	9
Smith Inlet	8
QC Strait	57
Kingcome Inlet	9
Knight Inlet	9
Tribune Inlet	8

**Option 2**

In Option 2 hauls are allocated to each stratum based on the 2003 data by minimizing the variance in abundance estimate for two areas: *combining Rivers/Smith Inlets and rest* (QC Strait/ Broughton Archipelago).

The following assumptions are made:

- Number of simulations – 10,000
- Maximum number hauls – 100
- Minimum number hauls in each stratum – 5

Table 2.

<b>Stratum</b>	<b>Minimum no. hauls</b>
Rivers Inlet	5
Smith Inlet	15
QC Strait	60
Kingcome Inlet	8
Knight Inlet	7
Tribune Inlet	5

\*This combination produces a lower overall variance than Option 1.

**Option 3**

A third option that combined information from Option 1 and 2 was also developed. This is the design that has been used in the Broughton region.

The following assumptions of option 3 are:

Maximum number hauls:

- Rivers/Smith Inlets – 20
- Broughton Archipelago – 10 hauls per inlet (x 3 inlets)

Table 3.

<b>Stratum</b>	<b>Minimum no. hauls</b>
Rivers Inlet	5
Smith Inlet	15
QC Strait	50
Kingcome Inlet	10
Knight Inlet	10
Tribune Inlet	10

### **Surface Hauls**

One hundred hauls are conducted at the surface. Any hauls above the daily goal of 10-hauls are conducted at depth. These additional hauls cover the depth stratum of 15-30-m. The additional hauls are used to verify the vertical distribution of juvenile pink and chum salmon in the QC Strait/ Broughton area. The additional hauls are made on an angle across the QC Strait or Inlets, and not parallel to the shoreline. Previous work in the Strait of Georgia has shown 95-99% of the catch of these species are in the surface layers.

In summary, the survey design used is a stratified systematic sampling (SSS) design that minimizes travel time/distance between tows, distribute effort allocation evenly over each stratum, and facilitates survey replication. The survey design has the following features:

- a preset track-line was used to ensure even distribution of effort throughout each stratum.
- tows are not placed parallel to shore; instead survey track-line are always kept on an angle. The same approach is taken for both the Inlets and the Strait.
- sampling intensity is distributed equally with respect to the shoreline. This removes biases associated with sampling intensity by ensuring each distance from the shore has an equal probability of being sampled, and by ensuring the survey is not sampling a cyclic pattern.

### **Analysis of Survey Data**

#### **The tow coordinates are imported into PBS Mapping for three purposes:**

1. To verify tow locations: in some cases, tow coordinates may appear to be incorrect and result in extremely long tows or tows on-land. These coordinates are adjusted to reflect average tow length (30 minutes at 5 knots).
2. To verify the allocation of set numbers to each stratum within the data set.
3. To recalculate stratum areas: stratum boundaries needed to be adjusted in order to accurately reflect tow locations. Table 4 summarizes the area calculations used for the July and September 2004 abundance estimates.

Table 4. Stratum areas used for abundance estimates calculations

	July 2004 area (km <sup>2</sup> )	September 2004 area (km <sup>2</sup> )
Rivers Inlet	328	328
Smith Inlet	179	179
QC Strait	1300	1300
Kingcome Inlet	125	168
Knight Inlet	309	309
Tribune Inlet	145	102

---

## Abundance estimates

Abundance estimates are calculated in two different ways:

1. using tow densities for each of the 6 strata independently.
2. combining tow densities into 3 strata: Stratum 1=Rivers+Smith Inlets, Stratum 2=QC Strait and Stratum 3=Broughton Inlets (Kingcome+Knight+Tribune Inlets).

Although it is possible to estimate juvenile densities for each stratum independently, several of the inlets have low sample sizes (an unavoidable limitation of the survey) and a high frequency of empty tows. Tighter confidence intervals can be achieved with larger sample sizes and for this survey this can be achieved by combining tow data from neighbouring inlets.

In order to combine tows from more than one area into a single stratum, the tow data must be standardized. This involved using a scaling factor so that the tow data from the individual strata are representative of the surveyed area.

For example, in combining juvenile pink salmon tow density data from Rivers and Smith Inlets the following calculations are used to scale the raw data from each stratum. This allows the data to be combined to calculate abundance estimates.

Calculate the composite sampling fraction (D):

$$D = \frac{\text{area of stratum } i}{\text{total area of all strata}} \times \frac{\text{total number tows in all strata}}{\text{tows in stratum } i}$$

Calculate scaled densities ( $S_n$ ) for stratum  $i$  by multiplying each tow by the composite sampling fraction:

$v_n$  = raw data for stratum  $i$  (vector of tow density data for stratum  $i$ )

$$S_n = v_{1:n} * D$$

Mean tow densities and abundance estimates for juvenile pink and chum salmon are calculated using the MLE method, and are presented in two ways:

1. calculated for each of the 6 strata independently, and
2. calculated for 3 strata, combining Rivers and Smith and combining Kingcome, Knight and Tribune Inlets.

## Maximum likelihood estimation (MLE)

The maximum likelihood estimation (MLE) procedure includes a binomial distribution for the zero observations (empty tows) and a lognormal distribution for the nonzero observations. Trawl survey data are typically described as being skewed with high variance, as well as having a high proportion of zero tows and one or several larger tows (up to 1000 fish per tow) which are highly influential (de la Mare 1994, Pennington 1996). Therefore assumptions regarding the underlying distribution of the nonzero data are useful when evaluating survey data, especially in the event of a low sample size. In contrast, the bootstrap estimation procedure makes no assumptions regarding the underlying distribution of survey data.

Simulation details:

1. The MLE method uses a lognormal distribution as the underlying statistical model for the nonzero data. It was important to determine whether the MLE method was robust to survey data with alternative underlying distributions (in the event that the survey data do not follow a lognormal distribution). Using simulations, a total of five distributions for the nonzero survey data were explored: lognormal, uniform, gamma, exponential, and inverse Gaussian. The

---

MLE model was applied to each of the simulated data sets in order to determine whether the MLE model is robust to different underlying distributions.

2. The following parameters were used for each experimental distribution:

Table 5. Population parameters for simulated distributions.

	Population 1	Population 2
<i>n</i>	50	10
<i>p</i> (0)	0.5	0.5
true mean	1,000	1,000
true SE	1.5275	1.5275
true CV	0.288	0.288
<i>n trials</i>	10,000	10,000

Note: *n trials* represents the number of Monte Carlo trials for each distribution.  
*p*(0) represents the probability of a zero tow (empty tow).

The parameters were held constant in order to facilitate direct comparison of the results from all 10 distributions (Pop1-lognormal, Pop2-lognormal, Pop1-uniform, Pop2-uniform...). In theory, over a large number of simulation trials (e.g., 10,000) the 95% confidence intervals should include the "true mean" 95% of the time. For each of the 10,000 trials the number of times the calculated confidence intervals did not include the "true mean" (from Table 5) and whether the "true mean" was lower than the lower 95% CI (model is overestimating abundance) or higher than the upper 95% CI (model is underestimating abundance) was recorded.

FINAL STEP: The bootstrap and MLE approaches were compared by determining the number of times the "true mean" was lower than the lower 95% CI ("*i*", model is overestimating abundance) or higher than the upper 95% CI ("*j*", model is underestimating abundance).

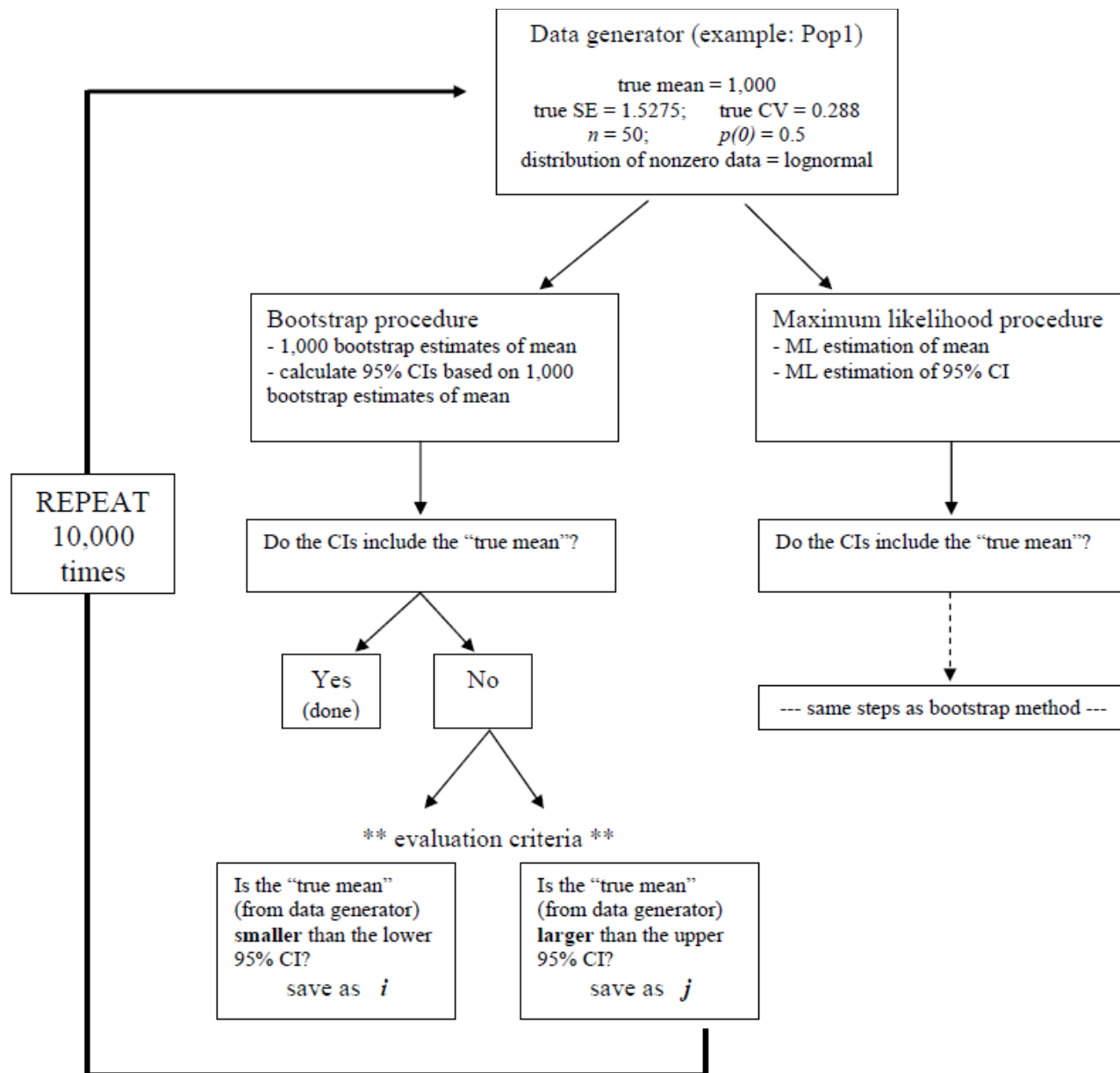


Figure 3. Diagram of stimulation study approach.

#### Simulation results

1. The MLE method produced abundance estimates which were accurate and unbiased when compared with “true values” for each set of simulated data (based on Table 5).
  - This means that the MLE procedure can be used for calculating abundance estimates regardless of the underlying distribution of the nonzero survey data.
2. The results of the simulation study comparing the MLE and bootstrap estimation procedures (via comparing CIs – see Figure 1) indicate the MLE approach is more robust than the bootstrap procedure.
  - Sample size = 50: CIs from 10,000 trials using the MLE approach include the “true mean” value more often than CIs from 10,000 trials using the bootstrap approach.

- 
- Sample size = 10: CIs from 10,000 trials using the MLE approach include the “true mean” value more often than CIs from 10,000 trials using the bootstrap approach.
- As sample size was reduced from  $n=50$  to  $n=10$  the MLE approach was increasingly more reliable than the bootstrap approach. This is important for your data as several inlets have low sample sizes ( $n \approx 10$ ).

### **References:**

- Beamish, R.J. and M. Folkes. 1998. Recent changes in the marine distribution of juvenile chum salmon in Canada. N. Pac. Anadr. Fish. Comm. Bull. 1:443-453.
- Beamish, R.J., D. McCaughran, J.R. King, R.M. Sweeting and G.A. McFarlane. 2000. Estimating the abundance of juvenile coho salmon in the Strait of Georgia by means of surface trawls. N. Am. J. Fish. Manage. 20:369-375.
- de la Mare, W. 1994. Estimating confidence intervals for fish stock abundance estimates from trawl surveys. CCAMLR Science. 1:203-207.
- Cochran, W.G. 1977. Sampling Techniques. Third Edition. John Wiley & Sons.
- Pennington, M. 1996. Estimating the mean and variance from highly skewed marine data. Fish. Bull. 94:498-505.



---

## **ADDENDUM 2: A BIBLIOGRAPHY OF SURVEY METHODS FOR SALMON ESCAPEMENT ENUMERATION**

**A draft document to categorize references describing salmon spawner enumeration according to field survey methods.**

Prepared by M. Stockwell, unpublished manuscript

Provided by Dr. K. Hyatt, DFO, Pacific Biological Station Nanaimo, B.C.

(Completed March 1999, revised Nov. 1999)

The following bibliography compiles references containing descriptions of salmon spawner enumeration techniques and attempts to categorize them according to field survey methods. References were obtained from published, annotated bibliographies (Cousens et. al. 1982 and Irvine & Nelson, 1995) and from a cursory literature search.

This bibliography was prepared as a source document to aid in the development of a Manual of Standardized Inventory Methodologies for Salmonid Spawner Abundance Surveys.

### ***Key References***

Cousens, N. B. F., G. A. Thomas, C. G. Swann and M. C. Healey. 1982. A review of salmon escapement enumeration techniques. Can. Tech. Rep. Fish. Aquat. Sci. 1108: 122 p.

Irvine, J. R. and T. C. Nelson (Eds). 1995. Proceedings of the 1994 salmon escapement workshop plus an annotated bibliography on escapement estimation. Can. Manuscr. Rep. Fish. Aquat. Sci. 2305: 97 p.

### ***Field Survey Methods for Enumeration of Salmon Spawners***

#### **A) FIXED SITE SURVEYS**

- 1) Fences
- 2) Fishways
- 3) Fixed Location Acoustics
- 4) Electronic Gates
- 5) Optical Gates
- 6) Fishwheels
- 7) Traps or Nets
- 8) Towers

#### **B) MOBILE SURVEYS**

##### **Aerial Surveys**

- 1) Fixed Wing Overflight
- 2) Helicopter Overflight
- 3) Remote Surveys

##### **Ground Surveys**

- 1) Streamwalk
- 2) Streamfloat – Above Surface Observer
- 3) Streamfloat – Below Surface Observer
- 4) Mobile Acoustics
- 5) Mark-Recapture Surveys
- 6) Interval Counts
- 7) Catch per Unit Effort Surveys

#### **C) PROXY SURVEYS**

- 1) Redd Surveys
- 2) Egg Surveys
- 3) Juvenile Surveys

---

## GENERAL REFERENCES

- Atkinson, C. E. 1944. The problem of enumeration of spawning populations of sockeye salmon. Int. Pac. Salmon Fish. Comm. Annu. Rep. 1943: 37-44.
- Fredd, L. C. 1966. Review and analysis of fish counts, counting techniques and related data at Corps of Engineers dams on the Columbia and Snake Rivers. U. S. Army Corps of Engineers, Fish. Eng. Res. Program Prog. Rep. 3: 91-95.
- Hilborn, R. 1983. Design of the B.C. salmon escapement monitoring system: notes from a workshop. Misc. Coop. Fish. Res. Unit Rep. 6: 25 p.
- Ricker, W. E. 1975. Computation and interpretation of biological statistics of fish populations. Bull. Fish. Res. Board. Can. 191: 382 p.
- Ricker, W. E. (Ed.). 1968. Methods for assessment of fish production in fresh waters. Int. Biol. Prog. Handbook 3: 313 p.
- Russell, L. R. 1975. An annotated bibliography on salmonid marking, enumeration and trapping techniques. B. C. Fish and Wildlife Branch Fish. Tech. Circ. 18: 194 p.
- Seber, G. A. F. 1982. The estimation of animal abundance and related parameters. Charles Griffin & Co. Ltd., London: xvii + 654 p.
- Smith, C. J. and P. Castle. 1994. Puget Sound chinook salmon (*Oncorhynchus tshawytscha*) escapement estimates and methods – 1991. NW Indian Fisheries Commission, Olympia, WA., NW Fishery Resource Bulletin Project Report Series 1:
- Straty, R. R. 1960. Methods of enumeration of salmon in Alaska. Trans. 25th N. Am. Wildl. Nat. Resour. Conf.: 286-297.
- Symons, P. E. K. and M. Waldichuk. 1984. Proceedings of the workshop on stream indexing for salmon escapement estimation, West Vancouver, B. C., 2-3 February 1984. Can. Tech. Rep. Fish. Aquat. Sci. 1326: 258 p.

## COMPARISON OF METHODS

- Andrew, J. H., G. D. Sutherland, and T. M. Webb. 1988. Abundance, age, size, sex and coded wire tag recoveries for chinook salmon escapements of Atnarko River, 1984-1986. Can. Manusc. Rep. Fish. Aquat. Sci. 2014: viii + 87 p.
- Beidler, W. M., T. E. Nickelson, and A. M. McGie. 1980. Escapement goals for coho salmon in coastal Oregon streams. Ore. DFW. R&D Inf. Rep. Ser., Fish. 80-10: 30 p.
- Bocking, R. C., J. R. Irvine, K. K. English, and M. Labelle. 1988. Evaluation of random and indexing sampling designs for estimating coho salmon (*Oncorhynchus kisutch*) escapement to three Vancouver Island streams. Can. Tech. Rep. Fish. Aquat. Sci. 1639: 95 p.
- Johnston, N. T., J. R. Irvine and J. C. Perrin. 1986. A comparative evaluation of fence count, mark-recapture and Bendix sonar estimates of salmon escapements in the Keogh River a variable-flow coastal B. C. stream. Can. Tech. Rep. Fish. Aquat. Sci. 1453: 44 p.
- Johnston, N. T., J. R. Irvine, and C. J. Perrin. 1987. Instream indexing of coho salmon (*Oncorhynchus kisutch*) escapement in French Creek, British Columbia. Can. Tech. Rep. Fish. Aquat. Sci. 1573: 37 p.
- McBride, D. and D. Mesiar. 1981. Nushagak sonar enumeration project, 1980. Alaska Dept. Fish Game Bristol Bay Data Rep. 83: 45 p.

- 
- Namtvedt, T. B., N. V. Friese, D. L. Waltemyer, M. L. Bethe and D. C. Whitmore. 1977. Investigations of Cook Inlet sockeye salmon. Alaska Dep. Fish Game. Tech. Rep. for period July 1, 1975 to June 30, 1976: 75 p.
- Robson, D. S. and H. A. Regier. 1968. Estimation of population number and mortality rates. Pages 124- 158, In: Ricker, W. E. (Ed.). 1968. Methods for assessment of fish production in fresh waters. Int. Biol. Prog. Handbook 3: 313 p.
- Tschaplinski, P. J. and K. D. Hyatt. 1990. Abundance, migration timing, and biological characteristics of sockeye salmon (*Oncorhynchus nerka*) returning to Henderson Lake, Vancouver Island during 1988. Can. Tech. Rep. Fish. Aquat. Sci. 1758: iv + 82 p.
- Tschaplinski, P. J. and K. D. Hyatt. 1991. A comparison of population assessment methods employed to estimate the abundance of sockeye salmon (*Oncorhynchus nerka*) returning to Henderson Lake, Vancouver Island during 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1798: 101 p.
- Zubik, R. J. and J. J. Fraley. 1988. Comparison of snorkel and mark-recapture estimates for trout populations in large streams. N. Am. J. Fish. Man. 8: 58-62.

## **A. FIXED SITE SURVEYS**

### **1) Fences**

- Aro, K. V. 1961. Summary of salmon enumeration and sampling data, Babine River counting weir, 1946 to 1960. Fish. Res. Board Can. Manusc. Rep. 708: 63 p.
- Bachen, S. K. , B. L. Thompson and R. D. Goruk. 1988. Docee River counting fence 1986 operations. Fish. Res. Board Can. Data Rep. 703: iv + 15 p.
- Clay, C. H. 1995. Design of fishways and other fish facilities. CRC Press, Inc., Boca Raton, Florida, U.S.A. 248 p.
- Clay, C. H. 1961. Design of fishways and other fish facilities. Department of Fisheries. Ottawa. 301 p.
- Cooke, K. D. and N. B. F. Cousens. 1981. Results of adult sockeye enumeration and sampling program 1980. J. C. Lee and Associates Ltd. Report Series 2-2: 167 p.
- Cousens, N. B. F., G. A. Thomas, C. G. Swann and M. C. Healey. 1982. A review of salmon escapement enumeration techniques. Can. Tech. Rep. Fish. Aquat. Sci. 1108: 122 p.
- Fraser River Sockeye Management Review Team, 1994 Spawning Escapement Estimation Working Group. 1995. Final report for early Stuart, early summer and summer runs.
- Hill, J. P. and W. J. Matter. 1991. A low-cost weir for salmon and steelhead. Prog. Fish Culturist 53: 255-258.
- Hunter, J. G. 1954. A weir for adult and fry salmon effective under conditions of extremely variable run-off. Can. Fish Cult. 16: 27-33.
- Johnston, N. T., J. R. Irvine and J. C. Perrin. 1986. A comparative evaluation of fence count, mark- recapture and Bendix sonar estimates of salmon escapements in the Keogh River a variable-flow coastal C. stream. Can. Tech. Rep. Fish. Aquat. Sci. 1453: 44 p.
- Lill, A. F. and Sookachoff. 1974. The Carnation Creek fish counting fence. Env. Can. Fish. Mar. Serv. Tech. Rep. Pac./T 74-2: 23 p.
- Mottraw, W. 1977. Design and construction of the Keogh River fish enumeration fence. Fish. Tech. Circ., B.C. Fish Wildl. 25: 18 p.

- 
- Nelson, P. R. 1960. Effects of fertilizing Bare Lake, Alaska, on growth and production of red salmon (*O. nerka*). U. S. Fish Wildl. Serv. Fish. Bull. 60(159): 59-86.
- Ruggles, C. P. 1980. Sampling migrating salmon. Pages 143-158, In: T. Backiel and R. L. Welcomme (eds.) Guidelines for sampling fish in inland waters. EIFAC Tech. Papers 33: 176 p.
- Wright, M. C. 1982. Results of adult sockeye enumeration and sampling program 1981. Section One. J. Lee and Associates Ltd. Report Series 2-4: 89 p.

## **2) Fishways**

- Clay, C. H. 1995. Design of fishways and other fish facilities. CRC Press, Inc., Boca Raton, Florida, U.S.A. 248 p.
- Clay, C. H. 1961. Design of fishways and other fish facilities. Department of Fisheries. Ottawa. 301 p.
- Cousens, N. B. F. 1982. Results of adult sockeye enumeration and sampling program 1981. Section two. J. C. Lee and Associates Ltd. Report Series 2-5: 187 p.
- Dunkley, D. A. 1984. An electronic fish counter. Scott. Fish. Bull. 48: 45-49.
- Jessop, B. M. and C. J. Harvie. 1990. Evaluation of designs of periodic count surveys for the estimation of escapement at a fishway. N. Am. J. Fish. Man. 10(1): 39-44.
- Johnson, D. R. 1948. Size of the Willamette River spring chinook salmon run, 1947. Oreg. Fish Comm. Res. Briefs 1(1): 18-21.
- Rich, W. H. 1942. The salmon runs of the Columbia River in 1938. U. S. Fish Wildl. Serv. Fish. Bull. 50(37): 103-147.
- Southgate, D. R., M. J. Jakubowski, V. L. Craig, and A. L. Jantz. 1988. Review of the Meziadin River fishway biological program for 1987 and 1988. Can. Data Rep. Fish. Aquat. Sci. 739: iii + 79 p.

## **3) Fixed Location Acoustics**

- Bendex Corporation. 1980. Installation and operation manual for side scan salmon counter. Bendex Corporation Oceanics Division Report SP-78-017, Sylmer, California.
- Braithwaite, H. 1971. A sonar fish counter. J. Fish Biol. 3(1): 73-82.
- Cheng, P., D. A. Levy, and P. A. Nealson. 1991. Hydroacoustic estimation of Fraser River pink salmon abundance and distribution at Mission, B.C., in 1987. Pacific Salmon Comm. Tech. Rep.3: iv + 35 p.
- Davis, A. S. 1968. Salmon counting by acoustic means. Alaska Dept. Fish Game Inf. Leaflet 113: 28 p.
- Eggers, D. M., P. A. Skvorc, II, and D. L. Burwen. 1995. Abundance estimates of chinook salmon in the Kenai River using dual-beam sonar. Alask. Fish. Res. Bull. 2(1): 1-22.
- Galloway, J. L. 1990. Sidescan stock assessment method. The Marine Survival of Salmon Program: Program Outline and Investigators Summaries for 1989/90, DFO Report Series, 1990.
- Gaudet, D. 1990. Enumeration of migrating salmon populations using fixed-location sonar counters. Rapp. P.-V. Reun. Cons. Explor. Mer. 189: 197-209.
- Hellawell, J. M. 1973. Automatic methods of monitoring salmon populations. Int. Atl. Salmon Found. Spec. Publ. Serv. 4(1): 317-337.

- 
- Johnson, S. V. and D. Daum. 1995. Escapement estimation of Chandalar River chum salmon with split- beam acoustic techniques. Pages 117-140, In: H. Fuss and G. Graves (eds.) Proceedings of the 17th Northeast Pacific pink and chum salmon workshop, March 1-3, 1995, Bellingham, Wash.
- King, B. E. 1989. Bendix Corporation 1984 model side-scanning sonar counter experiments in the Kenai River, 1986. Alask. Dept. Fish Game Tech. Fish. Rep. 89-04: 37 p.
- McBride, D. and D. Mesiar. 1981. Nushagak sonar enumeration project, 1980. Alaska Dept. Fish Game Bristol Bay Data Rep. 83: 45 p.
- Miller, J. D., L. K. Brannian, and B. A. Cross. 1994. Sonar enumeration of Pacific salmon into Nushagak River and evaluation of species composition estimates, 1992. Alask. Dep. Fish Game Tech. Fish. Rep. 94-06: 75 p.
- Namtvedt, T. B., N. V. Friese and D. L. Waltemyer. 1979. Cook Inlet sockeye salmon studies. Alaska Dep. Fish Game. Tech. Rep. for period July 1, 1977 to June 30, 1978: 41 p.
- Namtvedt, T. B., N. V. Friese, D. L. Waltemyer, M. L. Bethe and D. C. Whitmore. 1977. Investigations of Cook Inlet sockeye salmon. Alaska Dep. Fish Game. Tech. Rep. for period July 1, 1975 to June 30, 1976: 75 p.
- Skalski, J. R., A. Hoffmann, B. H. Ransom and T. W. Steig. 1993. Fixed-location hydroacoustic monitoring designs for estimating fish passage using stratified random and systematic sampling. Can. J. Fish. Aquat. Sci. 50: 1208-1221.
- Tarbox, K. E., B. E. King and D. L. Waltemyer. 1981. Cook Inlet sockeye salmon studies. Alaska Dep. Fish Game. Tech. Rep. for period July 1, 1979 to June 30, 1980: 101 p.
- Trevorrow, M. V. 1997. Detection of migrating salmon in the Fraser River using 100-kHz sidescan sonars. Can. J. Fish. Aquat. Sci. 54: 1619-1629.

#### **4) Electronic Gates**

- Beaumont, W. R. C., C. A. Mills, and G. I. Williams. 1986. Use of a microcomputer as an aid to identifying objects passing through a resistivity fish counter. Aquacult. Fish. Man. 17(3): 213-226.
- Bell, W. H. and M. C. Armstrong. 1970. A photo-electric fish counter. Fish. Res. Board Can. Tech. Rep. 215: 23 p.
- Brannian, L. K., S. J. Fleischman, J. D. Miller, and B. A. Cross. 1995. Evaluation of escapement estimation procedures for Pacific salmon into the Nushagak River as applied to the 1991 run. Alask. Dep. Fish Game Tech. Fish. Rep.
- Cheng, P., D. A. Levy, and P. A. Nealson. 1991. Hydroacoustic estimation of Fraser River pink salmon abundance and distribution at Mission, B.C., in 1987. Pacific Salmon Comm. Tech. Rep.3: iv + 35 p.
- Cousens, N. B. F. 1982. Results of adult sockeye enumeration and sampling program 1981. Section two. J. C. Lee and Associates Ltd. Report Series 2-5: 187 p.
- Dunkley, D. A. 1984. An electronic fish counter. Scott. Fish. Bull. 48: 45-49.
- Dunkley, D. A. and W. M. Shearer. 1982. An assessment of the performance of a resistivity fish counter. J. Fish. Biol. 20(6): 717-737.
- Dunkley, D. A. and W. M. Shearer. 1987. The freshwater environment and salmon stocks: The estimation of salmon stocks using electronic fish counters. Pages 197-206, In: Bailey, R. S. and B. B. Parrish (Eds.) Dev. Fish. Res. Scot.

- 
- Dunkley, D. A. and R. G. J. Shelton. 1991. Recent developments in automatic fish counters for salmon rivers. ICES Copenhagen: 12p.
- Gosset, C. 1986. Description and test of a resistivity fish counter. Bull. Fr. Peche-Piscic. 303: 141-151.
- Hellawell, J. M. 1973. Automatic methods of monitoring salmon populations. Int. Atl. Salmon Found. Spec. Publ. Serv. 4(1): 317-337.
- Kristinsson, B. and M. Alexanderdottir. 1978. Design and calibration of a salmon counter. J. Agr. Res. Icel. 10(2): 57-66. Ref #: 2-103
- Mann, R. H. K., J. M. Hellawell, W. R. C. Beaumont, and G. I. Williams. 1983. Records from the automatic fish counter on the River Frome, Dorset 1970-1981. Occas. Publ. Freshwat. Biol. Assoc. 19: 100 p.
- Morley, R. B. 1981. Adult sockeye escapements to Sproat Lake and Great Central Lake (Somass River System, Port Alberni, B. C.) in 1979. Can. Manusc. Rep. Fish. Aquat. Sci. 1614: v + 68 p.
- Reddin, D. G., M. F. O'Connell, and D. A. Dunkley. 1992. Assessment of an automated fish counter in a Canadian river. Aquacult. Fish. Man. 23(1): 113-121.
- Ruggles, C. P. 1980. Sampling migrating salmon. Pages 143-158, In: T. Backiel and R. L. Welcomme (eds.) Guidelines for sampling fish in inland waters. EIFAC Tech. Papers 33: 176 p.
- Tobin, J. H. 1994. Construction and performance of a portable resistance board weir for counting migrating adult salmon in rivers. Alaska Fish. Tech. Rept. 22: iii + 27 p.

#### **5) Optical Gates**

- Beach, M. H. 1978. The use of infra-red light and closed circuit TV to validate records from automatic fish counters. J. Fish. Biol. 13(5): 639-644.
- Gudjonsson, S. and H. Gudmundsson. 1994. Development and testing of a new light gate fish counter in rivers. ICES 1994 Stat. Meeting. C.M. 1994. ANACAT Comm. M. 14: 10 p.
- Hatch, D. R., M. Schwartzberg, and P. R. Mundy. 1994. Estimation of Pacific salmon escapement with a time-lapse video recording technique. N. Am. J. Fish. Man. 14: 626-635.
- Hatch, D. R., A. Wand, and M. Schwartzberg. 1992. Wenatchee River salmon escapement estimates using video tape technology in 1991. Columbia River Inter-Tribal Fish Commission, Tech. Rep. 92-3: 28 p.
- Pippy, J. H. C., W. G. Whelan, and M. F. O'Connell. 1997. A field guide to counting and measuring salmonids using the silhouette imaging and counting system (SIACS). Can. Manusc. Rep. Fish. Aquat. Sci. 2386: xi + 88 p.

#### **6) Fishwheels**

- Barton, L. H. 1992. Tanana River, Alaska, fall chum salmon radio telemetry study. Alask. Dept. Fish Game Fish. Res. Bull. 92-01: vii + 22 p.
- Donaldson, I. J. and F. K. Cramer. 1971. Fishwheels of the Columbia. Binfords and Mort, Portland, OR.

- 
- Koski, W. R., R. F. Alexander and K. K. English. 1994. Distribution, timing, fate and numbers of chinook salmon returning to the Nass River watershed in 1993. Report prepared by LGL Limited, Sidney, B.C., for the Nisga's Tribal Council, New Aiyansh, B.C.
- Link, M. R., and K. K. English. 1994. The 1993 fishwheel project on the Nass River and an evaluation of fishwheels as an inseason management and stock assessment tool for the Nass River. Can. Tech. Rep. Fish. Aquat. Sci. 2130: xi + 103 p.
- Link, M. R., K. K. English, and R. C. Bocking. 1996. The 1992 fishwheel project on the Nass River and an evaluation of fishwheels as an inseason management and stock assessment tool for the Nass River. Can. Manuscr. Rep. Fish. Aquat. Sci. 2372: x + 82 p.
- Link, M. R., and R. M. Peterman. 1998. Estimating the value of in-season estimates of abundance of sockeye salmon (*Oncorhynchus nerka*). Can. J. Fish. Aquat. Sci. 55(6): 1408-1418.
- Meehan, W. R. 1961. Use of a fishwheel in salmon research and management. Trans. Amer. Fish. Soc. 90: 490-494.
- Nagtegal, D. A., E. W. Carter, and D. C. Key. 1998. Results of the chinook assessment study conducted on the Klinaklini River during 1997. Can. Manuscr. Rep. Fish. Aquat. Sci. 2452: 59 p.

#### **7) Traps or Nets**

- Anderson, T. C. and B. P. MacDonald. 1978. A portable weir for counting migrating fish in rivers. Can. Fish. Mar. Serv. Tech. Rpt. 733: iv + 13 p.
- Hallock, R. J., D. F. Fry, Jr. and D. A. Lafaunce. 1957. The use of wire fyke traps to estimate the runs of adult salmon and steelhead in the Sacramento River. Calif. Fish Game 43(4): 271-298.
- Palmisano, A. N. and C. V. Burger. 1988. Use of a portable electric barrier to estimate chinook salmon escapement in a turbid Alaskan river. N. Am. J. Fish. Man. 8(4): 475-480.
- Sumner, F. H. 1953. Migrations of salmonids in Sand Creek, Oregon. Trans. Am. Fish. Soc. 82: 139-150.
- Tait, N., J. L. Hout and F. V. Thorsteinson. 1962. An evaluation of fyke trapping as a means of indexing salmon escapements in turbid streams. U. S. Fish Wildl. Serv. Spec. Sci. Rep. Fish. 428: 1-18
- Whelan, W. G., M. F. O'Connell and R. N. Hefford. 1989. Improved trap design for counting migrating fish in rivers. N. Am. J. Fish. Man. 9(2): 245-248.

#### **8) Towers**

- Becker, C. D. 1962. Estimating red salmon escapements by sample counts from observation towers. U. S. Fish Wildl. Serv. Fish. Bull. 61(192): 355-369.
- Cousens, N. B. F., G. A. Thomas, C. G. Swann and M. C. Healey. 1982. A review of salmon escapement enumeration techniques. Can. Tech. Rep. Fish. Aquat. Sci. 1108: 122 p.
- Gilbert, J. R. 1968. Surveys of sockeye salmon spawning populations in the Nushagak District, Bristol Bay, Alaska, 1946-1958. Pages 199-267, In: Burgner, R. L. 1968. Further studies of Alaska sockeye salmon. Univ. Wash. Publ. Fish. New Ser. 3: 267 p.
- Mathisen, O. A. 1962. Photographic enumeration of red salmon escapement. Pages 349-372, In: Koo, T.

- 
- S. Y. 1962. Studies of Alaska red salmon. U. of Wash. Pub. Fish. New Series Vol. 1.
- Morley, R. B. 1981. Adult sockeye escapements to Sproat Lake and Great Central Lake (Somass River system, Port Alberni, B. C.) in 1979. Can. Manuscr. Rep. Fish Aquat. Sci. 1614: 68 p.
- Seibel, M. C. 1967. The use of expanded ten-minute counts as estimates of hourly salmon migration past counting towers on Alaskan Rivers. Alaska Dept. Fish Game Inf. Leaflet 101: 35 p.

### **C) MOBILE SURVEYS**

#### **Aerial Surveys (Unidentified Aircraft Type)**

- Cousens, N. B. F., G. A. Thomas, C. G. Swann and M. C. Healey. 1982. A review of salmon escapement enumeration techniques. Can. Tech. Rep. Fish. Aquat. Sci. 1108: 122 p.
- Nelson, M. L. 1965. Red salmon spawning ground surveys in the Nushagak and Togiak Districts, Bristol Bay, 1965. Alaska Dep. Fish Game Inf. Leaflet 84: 40 p.
- West, I. F. and R. H. Goode. 1987. Aerial counts of spawning chinook salmon (*Oncorhynchus tshawytscha*) on the Rakaia River system, Canterbury, New Zealand, 1973-76. N.Z. J. Mar. Freshwater Res. 21: 563-572.

#### **1) Fixed Wing Overflight**

- Bevan, D. E. 1961. Variability in aerial counts of spawning salmon. J. Fish. Res. Board Can. 18(3): 337- 348.
- Jones, E. L., III, T. J. Quinn, II, and B. W. Van Alen. 1998. Observer accuracy and precision in aerial and foot survey counts of pink salmon in a southeast Alaska stream. N. Am. J. Fish. Manag. 18: 832-846.
- Nelson, M. L. 1986. Aerial survey instruction manual for Nushagak and Togiak District salmon and herring fisheries. Alask. Dept. Fish Game, Bristol Bay Data Rep. No. 86-11: 47 p.
- Nelson, M. L. 1979. Spawning ground surveys in the Nushagak and Togiak Districts of Bristol Bay, 1977- 1979. Alask. Dept. Fish Game, Bristol Bay Data Rep. No. 75: 55 p.
- Nelson, M. L. 1965. Red salmon spawning ground surveys in the Nushagak and Togiak Districts, Bristol Bay, 1965. Alaska Dep. Fish Game Inf. Leaflet 84: 40 p.
- Pirtle, R. B. 1977. Historical pink and chum salmon estimated spawning escapements from Prince William Sound, Alaska streams, 1960-1975. Alaska Dep. Fish Game Tech. Rep. 35: 332 p.
- Pirtle, R. B. and M. L. McCurdy. 1980. Prince William Sound General Districts 1976 pink (*Oncorhynchus gorbuscha*) and chum salmon (*O. keta*) aerial and ground escapement surveys and consequent brood year egg deposition and pre-emergent fry index programs. Alask. Dept. Fish Game Tech. Data Rep. 51: 62 p.

#### **2) Helicopter Overflight**

- Cooke, K. D. and N. B. F. Cousens. 1981. Results of adult sockeye enumeration and sampling program 1980. J. C. Lee and Associates Ltd. Report Series 2-2: 167 p.
- Eicher, G. J. 1953. Aerial methods of assessing red salmon populations in western Alaska. J. Wildl. Manage. 17(4): 521-527.



- 
- Jaremovic, L. and D. Rowland. 1988. Review of chinook salmon escapements in the Nechako River, British Columbia. Can. Manusc. Rep. Fish. Aquat. Sci. 1963: 135 p.
- Kelez, G. B. 1947. Measurement of salmon spawning by means of aerial photography. Pac. Fisherman 45: 46-51.
- Koski, W. R., M. R. Link, and K. K. English. 1996. Distribution, timing, fate, and numbers of chinook salmon returning to the Nass River watershed in 1992. Can. Tech. Rep. Fish. Aquat. Sci. 2129: 141 p.
- Neilson, J. D. and G. H. Geen. 1981. Enumeration of spawning salmon from spawner residence time and aerial counts. Trans. Am. Fish. Soc. 110: 554-556.

## **Ground Surveys**

### **1) Streamwalk**

- Bocking, R. C., J. R. Irvine, K. K. English, and M. Labelle. 1988. Evaluation of random and indexing sampling designs for estimating coho salmon (*Oncorhynchus kisutch*) escapement to three Vancouver Island streams. Can. Tech. Rep. Fish. Aquat. Sci. 1639: 95 p.
- Cooke, K. D. and N. B. F. Cousens. 1981. Results of adult sockeye enumeration and sampling program 1980. J. C. Lee and Associates Ltd. Report Series 2-2: 167 p.
- Cousens, N. B. F. 1983. Results of adult salmon enumeration and sampling program, Henderson Lake, 1982. J. C. Lee and Associates Ltd. Report Series 3-3: 55 p.
- Cousens, N. B. F., G. A. Thomas, C. G. Swann and M. C. Healey. 1982. A review of salmon escapement enumeration techniques. Can. Tech. Rep. Fish. Aquat. Sci. 1108: 122 p.
- Labelle, M. 1990. A comparative study of coho salmon populations of S.E. Vancouver Island, British Columbia: juvenile outmigration, coded-wire tagging and recovery, escapement enumeration, and stock composition at Black Creek, Trent River and French Creek, 1984-1988. Can. Tech. Rep. Fish. Aquat. Sci. 1722: vi + 148 p.
- Morrell, M. 1995. 1994 Spawner enumeration in the Clayoquot River system. Report (31-July-1995) prepared for the Clayoquot Biosphere Project, Tofino, B. C. 13 p.
- Pirtle, R. B. 1977. Historical pink and chum salmon estimated spawning escapements from Prince William Sound, Alaska streams, 1960-1975. Alaska Dep. Fish Game Tech. Rep. 35: 332 p.
- Sheridan, W. L. 1962. Variability in pink salmon escapements estimated from surveys on foot. U. S. Fish Wildl. Serv. Spec. Sci. Rep. Fish. 408: 7 p.
- Wright, M. C. 1982. Results of adult sockeye enumeration and sampling program 1981. Section One. J. C. Lee and Associates Ltd. Report Series 2-4: 89 p.

### **2) Streamfloat – Above Surface Observer**

- Bocking, R. C., K. K. English, J. R. Irvine, and M. Labelle. 1993. Statistical design and execution of an escapement estimation procedure for coho salmon in the Trent River, Vancouver Island. Pages 84-94, In: L. Berg and P. W. Delaney (Eds.) Proceedings of the Coho Workshop, Nanaimo, B. C., May 26-28, 1992.
- Cooke, K. D. and N. B. F. Cousens. 1981. Results of adult sockeye enumeration and sampling program 1980. J. C. Lee and Associates Ltd. Report Series 2-2: 167 p.

- 
- Cousens, N. B. F., G. A. Thomas, C. G. Swann and M. C. Healey. 1982. A review of salmon escapement enumeration techniques. *Can. Tech. Rep. Fish. Aquat. Sci.* 1108: 122 p.
- Hillman, T. W., J. W. Mullan and J. S. Griffith. 1992. Accuracy of underwater counts of juvenile chinook salmon, coho salmon and steelhead. *N. Amer. J. Fish. Manage.* 12: 598 – 603.
- Wright, M. C. 1982. Results of adult sockeye enumeration and sampling program 1981. Section One. J. C. Lee and Associates Ltd. Report Series 2-4: 89 p.
- Zubik, R. J. and J. J. Fraley. 1988. Comparison of snorkel and mark-recapture estimates for trout populations in large streams. *N. Am. J. Fish. Man.* 8: 58-62.

### **3) Streamfloat – Below Surface Observer**

- Cooke, K. D. and N. B. F. Cousens. 1981. Results of adult sockeye enumeration and sampling program 1980. J. C. Lee and Associates Ltd. Report Series 2-2: 167 p.
- Cousens, N. B. F., G. A. Thomas, C. G. Swann and M. C. Healey. 1982. A review of salmon escapement enumeration techniques. *Can. Tech. Rep. Fish. Aquat. Sci.* 1108: 122 p.
- Gardiner, W. R. 1984. Estimating population densities of salmonids in deep water streams. *J. Fish Biol.* 24: 41-49.
- Hankin, D. G. and G. H. Reeves. 1988. Estimating total fish abundance and total habitat area in small streams based on visual estimation methods. *Can. J. Fish. Aquat. Sci.* 45(5): 834-844.
- Helfman, G. S. 1983. Underwater methods. Pages 349-369, In: L. A. Nielsen and D. L. Johnson (eds.) 1983. *Fisheries Techniques*. American Fisheries Society, Southern Printing Co., Inc. 468 p.
- Keast, A., and J. Harker. 1977. Strip counts as a means of determining densities and habitat utilization patterns in lake fishes. *Environ. Biol. Fishes* 1: 181-188.
- Northcote, T. G. and D. W. Wilkie. 1963. Underwater census of stream fish populations. *Trans. Am. Fish. Soc.* 92: 146-151.
- Schill, D. J. and J. S. Griffith. 1984. Use of underwater observations to estimate cutthroat trout abundance in the Yellowstone River. *N. Am. J. Fish. Manage.* 4: 479-487.

### **4) Mobile Acoustics**

- Banneheka, S., R. Routledge, I. Guthrie, and J. Woodey. 1995. Estimation of in-river fish passage using a combination of transect and stationary hydroacoustic sampling. *Can. J. Fish. Aquat. Sci.* 52: 335-343.
- Braithwaite, H. 1971. A sonar fish counter. *J. Fish. Biol.* 3(1): 73-82
- Dahl, P. H. and O. A. Mathison. 1984. Some experiments and considerations for development of Doppler-based riverine sonars. *IEEE J. Ocean. Eng. OE-9(3)*: 214-217.
- Goruk, R. D. and B. L. Thomson. 1988. Rivers Inlet echo sounding program, 1967-1988. *Can. Manusc. Rep. Fish. Aquat. Sci.* 1989: 30 p.
- Hyatt, K. D., J. Candy, M. Wright, and D. P. Rankin. 1990. An appraisal of the potential utility of hydroacoustic techniques for routine estimation of sockeye salmon (*Oncorhynchus nerka*) escapements in British Columbia lakes. *Int. Symp. Fish. Acoustics, Seattle, 22-26 Jun 1987*; 189: 425.
- Mulligan, T. J. and R. Keiser. 1986. Comparison of acoustic population estimates of salmon in a lake with a weir count. *Can. J. Fish. Aquat. Sci.* 43(7): 1373-1385.

---

Thorne, R. E. 1979. Hydroacoustic estimates of adult sockeye salmon (*Oncorhynchus nerka*) in Lake Washington, 1972-75. J. Fish. Res. Board Can. 36: 1145-1149.

Vroom, P. R. 1971. An attempt to determine abundance and distribution of migrating Skeena River salmon stocks by acoustical means. Can. Dep. Fish. For., Fish Serv. Pac. Reg. Tech. Rep. 71-5: 33 p.

## 5) Mark-Recapture Surveys

Adair, R. A. and D. L. Cole. 1977. Population estimation of the 1976 fall chinook runs in the Duwamish - Green River and the Lake Washington watershed. Muckleshoot Indian Tribe and U. S. Fish and Wildlife Service Fisheries Assistance Office, Olympia, Washington. Preliminary Report: 12 p.

Andrew, J. H., G. D. Sutherland, and T. M. Webb. 1988. Abundance, age, size, sex and coded wire tag recoveries for chinook salmon escapements of Atnarko River, 1984-1986. Can. Manuscr. Rep. Fish. Aquat. Sci. 2014: viii + 87p.

Anon. 1956. Investigations and field studies relating to numbers and seasonal occurrence of migratory fish entering the Columbia River above Bonneville and the Snake River and their final distribution among principal tributaries thereto. U. S. Army Corps of Engineers, Fish. Eng. Res. Program Prog. Rep.: 104-111.

Bevan, E. B. 1962. Estimation by tagging of the size of migrating salmon populations in coastal waters. Pages 377-449, In: Koo, T.S.Y. 1962. Studies of Alaska red salmon. Univ. Wash. Publ. in Fish. New Ser. 1: 449 p.

Buklis, L. S. 1981. Yukon and Tanana River fall chum salmon tagging study 1976-1980. Alaska Dep. Fish Game Inf. Leaflet 194: 40 p.

Conrad, R. H., R. A. Hayman, and E. Beamer. 1988. Coho salmon escapement to the Skagit River estimated using a mark-recapture method: 1988. NW Indian Fisheries Commission, Olympia, WA., NW Fishery Resource Bulletin Project Report Series 8: viii + 70 p.

Cousens, N. B. F. 1982. Results of adult sockeye enumeration and sampling program 1981. Section two. J. C. Lee and Associates Ltd. Report Series 2-5: 187 p.

Cousens, N. B. F., G. A. Thomas, C. G. Swann and M. C. Healey. 1982. A review of salmon escapement enumeration techniques. Can. Tech. Rep. Fish. Aquat. Sci. 1108: 122 p.

Eames, M. and M. Hino. 1981. A mark-recapture study of an enumerated coho spawning escapement. Wash. Dep. Fish. Prog. Rep. 148: 22 p.

Eames, M., T. Quinn, K. Reidinger and D. Haring. 1981. Northern Puget Sound 1976 adult coho and chum tagging studies. Wash. Dep. Fish. Tech. Rep. 64: 217 p.

Fraser River Sockeye Management Review Team, 1994 Spawning Escapement Estimation Working Group. 1995. Final report for early Stuart, early summer and summer runs.

Frith, H. R. and T. C. Nelson. 1994. Abundance, age, size, sex and coded wire tag recoveries for chinook salmon escapements of Campbell and Quinsam rivers, 1993. Can. Manuscr. Rep. Fish. Aquat. Sci. 2251: ix + 59 p.

Howard, G. V. 1948. A study of the tagging method in the enumeration of sockeye salmon populations. Int. Pac. Salmon Fish. Comm. Bull. 11: 9-66.

Johnston, N. T., J. R. Irvine and J. C. Perrin. 1986. A comparative evaluation of fence count, mark-recapture and Bendix sonar estimates of salmon escapements in the Keogh River a variable-flow coastal C. stream. Can. Tech. Rep. Fish. Aquat. Sci. 1453: 44 p.

- 
- Kruse, T. E. 1964. A comparison of spaghetti and Petersen tags used on steelhead trout at Gnat Creek, Oregon. *Oreg. Fish Comm. Res. Briefs* 10(1): 57-66.
- Labelle, M. 1990. A comparative study of coho salmon populations of S.E. Vancouver Island, British Columbia: juvenile outmigration, coded-wire tagging and recovery, escapement enumeration, and stock composition at Black Creek, Trent River and French Creek, 1984-1988. *Can. Tech. Rep. Fish. Aquat. Sci.* 1722: vi + 148 p.
- Morgan, A. R. and K. A. Henry. 1959. The 1955-1956 silver salmon run into the Tenmile Lakes system. *Oreg. Fish. Comm. Res. Briefs* 7(1): 57-77.
- Nelson, T. C. 1994. Abundance, age, size, sex and coded wire tag recoveries for chinook salmon escapement of Kitsumkalum River, 1993. *Can. Manuscr. Rep. Fish. Aquat. Sci.* 2249: viii + 47 p.
- Parker, R. R. and W. Kirkness. 1954. Estimates of spawning king salmon in the Taku River, Alaska, for the year 1951. Pages 179-191, In: Anon. 1954. Proceedings of the Third Alaskan Science Conference, 1952. *Alaska Div. Am. Assoc. Adv. Sci.*: 221 p.
- Pirtle, R. 1956. Enumeration study Upper Columbia and Snake Rivers. U. S. Army Corps of Engineers, Fish. Eng. Res. Program, Prog. Rep. Nov. 1956: 112-116.
- Pritchard, A. L. 1945. Sockeye salmon tagging of the Skeena River in 1945. *Fish. Res. Board Can. Prog. Rep. Pac. Coast Sta.* 65: 77-79.
- Pritchard, A. L. 1947. The use of a tagging ratio to estimate escapement, Babine fence 1946. *Fish. Res. Board Can. Manuscr. Rep.* 531: 17 p.
- Pritchard, A. L. and F. Neave. 1942. What did the tagging of coho salmon at Skutz Falls, Cowichan River, reveal? *Fish. Res. Board Can. Prog. Rep. Pac. Coast Sta.* 51: 8-11.
- Pritchard, A.L. 1947. Attempts to employ tagging in estimation of salmon spawning populations. *Fish Res Board Can. Manuscr. Rep.* 533: 17 p.
- Schaefer, M. B. 1951. A study of the spawning populations of sockeye salmon in the Harrison River system, with special reference to the problem of enumeration by means of marked members. *Int. Pac. Salmon Fish. Comm. Bull.* 4: 207 p.
- Schubert, N. D., M. K. Farwell and L. W. Kalnin. 1994. Enumeration of the 1993 Harrison River chinook salmon escapement. *Can. Manuscr. Rep. Fish. Aquat. Sci.* 2242: 27 p.
- Schubert, N. D., M. K. Farwell, and L. W. Kalnin. 1994. A coded wire tag assessment of Salmon River (Langley) coho salmon: 1992 tag application and 1993-1994 spawner enumeration. *Can. Manuscr. Rep. Fish. Aquat. Sci.* 2241: 33 p.
- Schubert, N. D., T. R. Whitehouse and A. J. Cass. 1997. Design and evaluation of the 1995 Fraser River pink salmon (*Oncorhynchus gorbuscha*) escapement estimation study. *Can. Tech. Rep. Fish. Aquat. Sci.* 2178: 75 p.
- Shardlow, T. F., T. M. Webb, and D. T. Lightly. 1986. Chinook salmon escapement estimation on the Campbell and Quinsam rivers in 1984: accuracy and precision of mark/recapture techniques using tagged salmon carcasses. *Can. Tech. Rep. Fish. Aquat. Sci.* 1507: viii + 52 p.
- Simpson, K. 1984. The accuracy of mark-recapture estimates of escapements. Pages 209-225, In: P. K. Symons and M. Waldichuk (Eds.) 1984. Proceedings of the workshop on stream indexing for salmon escapement estimation, West Vancouver, B.C., 2 - 3 February, 1984. *Can. Tech. Rep. Fish. Aquat. Sci.* No. 1326: xv + 258 p.

- 
- Stott, B. 1968. Marking and tagging. Pages 78-92, In: Ricker, W. E. (Ed.) 1968. Methods for assessment of fish production in fresh waters. Int. Biol. Prog. Handbook 3: 313 p.
- Tschaplinski, P. J. and K. D. Hyatt. 1990. Abundance, migration timing, and biological characteristics of sockeye salmon (*Oncorhynchus nerka*) returning to Henderson Lake, Vancouver Island during 1988. Can. Tech. Rep. Fish. Aquat. Sci. 1758: iv + 82 p.
- Willis, R. A. 1954. Population limits of the silver salmon run in Tillamook Bay during the 1951 fishing season. Oreg. Fish. Comm. Res. Briefs 5(1): 3-7.
- Winther, I. 1993. 1992 Wannock River chinook salmon mark-recapture experiment. Can. Manusc. Rep. Fish. Aquat. Sci. 2188: iv + 56 p.
- Wright, M. C. 1982. Results of adult sockeye enumeration and sampling program 1981. Section One. J. Lee and Associates Ltd. Report Series 2-4: 89 p.
- Wydoski, R. and L. Emery. 1983. Tagging and marking. Pages 215-237, In: L. A. Nielsen and D. L. Johnson (eds.) 1983. Fisheries Techniques. American Fisheries Society, Southern Printing Co., Inc. 468 p.

## 6) Interval Counts

- Bocking, R. C., J. R. Irvine, K. K. English, and M. Labelle. 1988. Evaluation of random and indexing sampling designs for estimating coho salmon (*Oncorhynchus kisutch*) escapement to three Vancouver Island streams. Can. Tech. Rep. Fish. Aquat. Sci. 1639: 95 p.
- Bucher, W. A. 1982. Spawning ground surveys in the Nushagak and Togiak districts of Bristol Bay, 1981. Alaska Dep. Fish Game Bristol Bay Data Rep. 87: 31 p.
- Cousens, N. B. F., G. A. Thomas, C. G. Swann and M. C. Healey. 1982. A review of salmon escapement enumeration techniques. Can. Tech. Rep. Fish. Aquat. Sci. 1108: 122 p.
- Crumley, L. and D. Pratt. 1977. The Lake Washington sockeye enumeration study at the Hiram M. Chittenden Ship Canal, 1972 through 1976. Wash. Dep. Fish. Prog. Rep. 23: 23 p.
- Flint, T. 1984. A comparison of stream indexing methods to estimate coho escapements in Harris Creek, 1980-1983. Pages 149-159, In: P. K. Symons and M. Waldichuk (eds.). 1984. Proceedings of the workshop on stream indexing for salmon escapement estimation, West Vancouver, B.C., 2 - 3 February, 1984. Can. Tech. Rep. Fish. Aquat. Sci. 1326: xv + 258 p.
- Gilbert, J. R. 1968. Surveys of sockeye salmon spawnig populations in the Nushagak District, Bristol Bay, Alaska, 1946-1958. Pages 199-267, In: Burgner, R. L. 1968. Further studies of Alaska sockeye salmon. Univ. Wash. Publ. Fish. New Ser. 3: 267 p.
- Irvine, J. R., R. C. Bocking, K. K. English, and M. Labelle. 1992. Estimating coho salmon (*Oncorhynchus kisutch*) spawning escapements by conducting visual surveys in areas selected using stratified random and stratified index sampling designs. Can. J. Fish. Aquat. Sci. 49(10): 1972-1981.
- Johnston, N. T., J. R. Irvine, and C. J. Perrin. 1987. Instream indexing of coho salmon (*Oncorhynchus kisutch*) escapement in French Creek, British Columbia. Can. Tech. Rep. Fish. Aquat. Sci. 1573: 37 p.
- McGie, A. M. 1981. Trends in the escapement and production of fall chinook and coho salmon in Oregon. Ore. Fish Div. Inf. Rep. 81-7: 44 p.

- 
- Mundie, J. H. 1984. Stream indexing: its usefulness and limitations. Pages 41-48, In: P. K. Symons and M. Waldichuck (eds.) 1984. Proceedings of the workshop on stream indexing for salmon escapement estimation, West Vancouver, B. C., 2 – 3 February, 1984. Can. Tech. Rep. Fish. Aquat. Sci. 1326: xv + 258 p.
- Solazzi, M. F. 1984. Relationships between visual counts of coho salmon (*Oncorhynchus kisutch*) from spawning fish surveys and the actual number of fish present. Pages 175-186, In: P. K. Symons and M. Waldichuk (eds.). 1984. Proceedings of the workshop on stream indexing for salmon escapement estimation, West Vancouver, B.C., 2 - 3 February, 1984. Can. Tech. Rep. Fish. Aquat. Sci. 1326: xv + 258 p.
- Tschaplinski, P. J. and K. D. Hyatt. 1990. Abundance, migration timing, and biological characteristics of sockeye salmon (*Oncorhynchus nerka*) returning to Henderson Lake, Vancouver Island during 1988. Can. Tech. Rep. Fish. Aquat. Sci. 1758: iv + 82 p. Ref #: 1-78
- Tschaplinski, P. J. and K. D. Hyatt. 1991. A comparison of population assessment methods employed to estimate the abundance of sockeye salmon (*Oncorhynchus nerka*) returning to Henderson Lake, Vancouver Island during 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1798: 101 p.
- Willis, R. A. 1964. Experiments with repeated spawning ground counts of coho salmon in three Oregon streams. Oreg. Fish. Comm. Res. Briefs 10(1): 41-45.

## **7) Catch per Unit Effort Surveys**

- Brannian, L. K., S. J. Fleischman, J. D. Miller, and B. A. Cross. 1995. Evaluation of escapement estimation procedures for Pacific salmon into the Nushagak River as applied to the 1991 run. Alask. Dept. Fish Game Tech. Fish. Rep. 95-01: 68 p.
- Cousens, N. B. F., G. A. Thomas, C. G. Swann and M. C. Healey. 1982. A review of salmon escapement enumeration techniques. Can. Tech. Rep. Fish. Aquat. Sci. 1108: 122 p.
- Cox-Rogers, S. and L. Jantz. 1993. Recent trends in the catchability of sockeye salmon in the Skeena River gillnet test fishery, and impacts on escapements estimation. Can. Man. Rep. Fish. Aquat. Sci. 2219: iii + 19 p.
- Gangmark, H. A. 1957. Fluctuations in abundance of Columbia River chinook salmon, 1928-54. U. S. Fish Wildl. Serv. Spec. Sci. Rep. Fish. 189: 21 pp.
- Gillhousen, P. 1992. Estimation of Fraser River sockeye escapements from commercial harvest data, 1892-1944. Int. Pac. Salmon Fish. Comm. Bull. 27: 114p.
- Gulland, J. A. 1964. Catch per unit effort as a measure of abundance. Pages 8-14, In: J. A. Gulland (Ed.) On the measurement of abundance of fish stocks. Rapp. P. V. Reun. Cons. Int. Explor. Mer. 155.
- Jantz, L., R. Kadowaki, and B. Spilsted. 1990. Skeena river salmon test fishery. Can. Data Rep. Fish. Aquat. Sci. 804: iv + 151p.
- Palmer, R. N. 1972. Fraser River chum salmon. Can. Dep. Env., Fish. Serv. Pac. Reg. Tech. Rep. 72-1: 284 p.
- Rounsefell, G. A. 1949. Methods of estimating total runs and escapements of salmon. Biometrics 5(2): 115-126.
- Silliman, R. P. 1950. Fluctuations in abundance of Columbia River chinook salmon (*Oncorhynchus tshawytscha*), 1935-1945. U. S. Fish Wildl. Serv. Fish. Bull. 51(51): 363-383.

- 
- Stratton, B. L. and J. D. Woolington. 1992. Bristol Bay sockeye salmon spawning escapement test fishing in 1991. Alask. Dept. Fish Game Tech. Fish. Rep. 92-15: vii + 69 p.
- Tait, N., J. L. Hout and F. V. Thorsteinson. 1962. An evaluation of fyke trapping as a means of indexing salmon escapements in turbid streams. U. S. Fish Wildl. Serv. Spec. Sci. Rep. Fish. 428: 1-18.
- Todd, I. S. and F. V. Dickson. 1970. Nass River sockeye salmon. A review of the commercial fishery and a summary of the 1963 to 1969 biological programs. Can. Dep. Fish. For., Fish. Serv. Pac. Reg. Tech. Rep. 70-10: 60 p.

## **C. PROXY SURVEYS**

### **1) Redd Surveys**

- Ames, J. and D. E. Phinney. 1977. Puget Sound summer-fall chinook methodology: escapement estimates and goals, run size forecasts and in-season run size up-dates. Wash. Dep. Fish. Tech. Rep. 29: 71 p.
- Beland, Kenneth F. 1996. The relation between redd counts and Atlantic salmon (*Salmo salar*) parr populations in the Dennys River, Maine. Can. J. Fish. Aquat. Sci. 53(3): 513-519.
- Conrad, R. H., R. A. Hayman, E. A. Beamer, and P. J. Goddard. 1993. Estimates of the number of coho salmon in the escapement to the Skagit River using a redd-count method: 1986-1990. Draft Report, Northwest Indian Fisheries Commission, Olympia, WA.: 112 p.
- Hay, D. W. 1984. The relationship between redd counts and the numbers of spawning salmon in the Girnock Burn (Scotland). ICES/C.W. No. M:22. International Council for the Exploration of the Sea, Charlottenlund, Denmark.
- Newman, K. 1984. Sampling strategies for estimating coho escapement. Pages 161-174, In: P. K. Symons and M. Waldichuk (Eds.) 1984. Proceedings of the workshop on stream indexing for salmon escapement estimation, West Vancouver, B.C., 2 - 3 February, 1984. Can. Tech. Rep. Fish. Aquat. Sci. No. 1326: xv + 258 p.
- Smith, C. J. and P. Castle. 1994. Puget Sound chinook salmon (*Oncorhynchus tshawytscha*) escapement estimates and methods – 1991. NW Indian Fisheries Commission, Olympia, WA., NW Fishery Resource Bulletin Project Report Series 1:

### **2) Egg Surveys Juvenile Surveys**

- Dementjeva, T. F. 1964. Correlations between indices of relative abundance of young fish, recruitment size and maturity rate as a basis for annual prediction. Rapp. P. V. Reun. Cons. Int. Explor. Mar. 155: 183-187.
- Holtby, B. 1995. Juvenile assessments as indices of escapement. (Abstract only) Pages 54-55, In: J. R. Irvine and T. C. Nelson (Eds.) Proceedings of the 1994 salmon escapement workshop plus an annotated bibliography on escapement estimation. Can. Manuscr. Rep. Fish. Aquat. Sci. 2305: 97 p.

### **3) Unknown / Other**

- Jacobs, S. E. 1989. Oregon coastal salmon spawning surveys. Ore. Fish Div. Prog. Rep.: 115 p.
- Jacobs, S. E. and C. X. Cooney. 1990. Improvement of methods used to estimate the spawning escapement of Oregon coastal natural coho salmon. Ore. Fish Div. Prog. Rep.: 17 p.

---

Johnson, B. A. and B. M. Barrett. 1988. Estimation of salmon escapement based on stream survey data. Alask. Dept. Fish Game, Div. of Com. Fish., Reg. Info. Rep. 4K88-35, Kodiak.

O'Connell, M. F. and J. B. Dempson. 1992. Status of Atlantic salmon (*Salmo salar* L.) in selected rivers with counting facilities in the Newfoundland Region. CAFSAC Res. Doc. 92/23: 20 p.