

**Relative abundance of farmed Atlantic salmon (*Salmo salar* L. 1758)  
juveniles in wild samples from three southwestern New Brunswick  
rivers**

by

Michael J. W. Stokesbury

Thesis

Submitted in partial fulfillment of the requirements for  
the Degree of Master of Science (Biology)

Acadia University

Spring Convocation 2000



National Library  
of Canada

Acquisitions and  
Bibliographic Services

395 Wellington Street  
Ottawa ON K1A 0N4  
Canada

Bibliothèque nationale  
du Canada

Acquisitions et  
services bibliographiques

395, rue Wellington  
Ottawa ON K1A 0N4  
Canada

*Your file* *Votre référence*

*Our file* *Notre référence*

The author has granted a non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of this thesis in microform, paper or electronic formats.

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

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

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

0-612-52002-1

**Canada**

## Table of Contents

Title Page.....	i
Approval Page.....	ii
Permission for Duplication Page.....	iii
Table of Contents.....	iv
List of Tables.....	vi
List of Figures.....	vii
Abstract.....	xiii
Acknowledgement Page.....	xiv
Dedication.....	xv
Introduction.....	1
<b>Effects of cultured rearing on salmonids.....</b>	<b>2</b>
<b>Identification of farmed or native origin.....</b>	<b>5</b>
Site Selection.....	10
Materials and Methods.....	13
<b>Samples.....</b>	<b>13</b>
<b>Applicability to Digdequash and Waweig unknown origin juveniles.....</b>	<b>15</b>
<b>Sampling Procedure.....</b>	<b>17</b>
<b>Temperature and pH profiles.....</b>	<b>17</b>
<b>Scale Analysis.....</b>	<b>18</b>
<b>Statistics .....</b>	<b>19</b>
Results.....	25

<b>Discriminate Function for Predicted Origin.....</b>	<b>25</b>
<b>Application of Discriminate Function to wild juveniles from the Magaguadavic River.....</b>	<b>26</b>
<b>Application of Discriminate Function to wild juveniles from the Digdequash River.....</b>	<b>26</b>
<b>Application of Discriminate Function to wild juveniles from the Waweig River.....</b>	<b>27</b>
<b>Temperature and pH.....</b>	<b>27</b>
<b>Discussion.....</b>	<b>28</b>
<b>Scale Characteristic Predictors of Origin.....</b>	<b>29</b>
<b>Implications of the Study.....</b>	<b>31</b>
<b>Limitations of the Study.....</b>	<b>34</b>
<b>Conclusions.....</b>	<b>37</b>
<b>References.....</b>	<b>38</b>
<b>Tables .....</b>	<b>52</b>
<b>Figures.....</b>	<b>57</b>
<b>Appendices.....</b>	<b>87</b>

### **List of Tables**

- 1) **Studies using scale characteristics (or data calculated from scale characteristics) as predictor variables in discriminant function analysis to classify Atlantic salmon to groups of natal origin or location..... 52**
- 2) **Source of Atlantic salmon juveniles from which scales were used for the discriminant function analysis..... 53**
- 3) **Results of a logistic regression performed on variables measured from scale characteristics of Atlantic salmon juveniles from the Magaguadavic River (N = 30) and Dennis Stream (N = 27), that were used to form the “Known wild” group used in the discriminant function analysis, B = the slope of the regression line. Measurements are for both river groups combined..... 54**
- 4) **Significance of predictors of group origin (hatchery or wild) of Atlantic salmon juveniles from the Magaguadavic River as computed by a discriminant function analysis using hatchery parr as a “known hatchery” group (N = 67) and wild parr from the Magaguadavic river (1996) and Dennis Stream (1998) as a “known wild” group (N = 57)..... 55**
- 5) **Results of the Discriminate function analysis performed on “unknown origin” juvenile salmon from the Magaguadavic River from 1996-1998; the Digdequash River 1998 and the Waweig River 1998.....56**

### **List of Figures**

- 1) Rivers of southwestern New Brunswick and Maine from which juvenile Atlantic salmon were sampled and smolt age statistics were used..... 57
  
- 2) Commercial Atlantic salmon aquaculture hatcheries on the Magaguadavic River system, southwestern New Brunswick; 1 = Stolt Sea Farms hatchery; 2 = Connors Brothers Ltd. hatchery; 3 = Tomlinson hatchery..... 58
  
- 3) Native origin juvenile Atlantic salmon removals from the Magaguadavic River, New Brunswick in 1998 to be used in the “known origin native” group to help compute the discriminate function (1 = lower Trout Brook; 2 = Kedron Stream; 3 = Pischahagen Stream)..... 59
  
- 4) Wild juvenile Atlantic salmon removals from the Magaguadavic River, New Brunswick in 1996..... 60
  
- 5) Wild juvenile Atlantic salmon removals from the Magaguadavic River, New Brunswick in 1997..... 61
  
- 6) Wild juvenile Atlantic salmon removals from the Magaguadavic River, New Brunswick in 1998 (1 = Tomlinson Corner site; 2 = Kedron Stream site; 3 = Linton Stream site)..... 62

- 7) **Scale from a 1-year-old Atlantic salmon taken from the Digdequash River, New Brunswick on 22 July 1998 and predicted to be of farmed origin by the discriminant function analysis. Reference line is shown on the longest axis of the scale from the center of the focus to the scales edge..... 63**
- 8) **Scale from a 1-year-old Atlantic salmon from the Waweig River, New Brunswick captured on 23 July 1998 and predicted to be of native origin by the discriminant function analysis. Scale shows Circuli pairs used in the discriminant function as predictors of origin..... 64**
- 9) **Scale taken from a 1-year-old Atlantic salmon on 27 January 1997 from Connors Brothers hatchery on the Magaguadavic River, N. B. Data from this scale was used in the formation of the “known origin farmed” sample for the discriminant function analysis. The area of the focus (inside of the first circuli) was generally larger for farmed salmon than for wild salmon; it was used as a predictor of farmed or native origin in the discriminate function analysis..... 65**
- 10) **Scale from a 0-year-old Atlantic salmon captured September 1997 at Tomlinson Corner, Magaguadavic River, N.B. This salmon was predicted as being of native origin by the discriminant function analysis. This scale has a degraded edge and would not be appropriate for evaluation by any method that required back-calculation of length..... 66**

- 11) Scale taken from a 1-year-old Atlantic salmon on 27 January 1997 from the Stolt Sea Farm Inc. hatchery on the Magaguadavic River, N. B. Data from this scale was used in the formation of the “known farmed origin” sample for the discriminant function analysis. Note the large and consistent distance between the first five circuli pairs..... 67
- 12) Scale taken from a 0-year-old Atlantic salmon from Tomlinson Corner, Magaguadavic River. This salmon was predicted to be of farmed origin by the discriminant function analysis. This is an example of the minimum amount of circuli formation that a salmon may have and still be evaluated to natal origin by this discriminant function analysis procedure..... 68
- 13) Scale from a 0-year-old Atlantic salmon captured 21 July 1998 in Linton Stream, Magaguadavic River N. B. This salmon was predicted as being of farmed natal origin by the discriminant function analysis. The circuli patterns indicate that this juvenile has undergone rapid, consistent growth..... 69
- 14) Scale from a 2-year-old Atlantic salmon captured in the lower Trout Brook of the Magaguadavic River N. B. Characteristics from this scale were used to compute the “known origin native” sample for the discriminant function analysis. At the second annulus there has been some adsorption of the scale reducing the number of the winter bands..... 70



- 15) Scale taken from a 2-year-old Atlantic salmon collected on 19 August 1998, from the Kedron Stream of the Magaguadavic River N. B. This salmon was predicted native by the discriminant function analysis. Note the close spacing of the first five circuli pairs..... 71
- 16) Scale from a 1-year-old Atlantic salmon taken from the Digdequash River, New Brunswick on 20 August 1998 and predicted to be of native origin by the discriminant function analysis. Note the inconsistent spacing of the first five circuli..... 72
- 17) Scale from a 1-year-old Atlantic salmon captured on 27 September 1998 in the Linton Stream, Magaguadavic River, N. B. This salmon was predicted as being of native origin by the discriminant function analysis. The large amount of growth in the 1+ year may be attributed to the high temperature of the Linton stream in 1998..... 73
- 18) Scale from a 2-year-old Atlantic salmon captured in the Dennis Stream, New Brunswick on 23 July 1998 and used to partly form the “known native origin” group for the discriminant function..... 74

- 19) **Scale from a 1-year-old Atlantic salmon captured in the Waweig River, New Brunswick on 23 July 1998 and predicted to be of farmed origin by the discriminant function analysis. Note the consistent circuli formation..... 75**
- 20) **Distribution of Known native (cross-hatched) farmed (black) and unknown origin (white) juvenile Atlantic salmon from the Magaguadavic River (1996-1998); as determined by a discriminant function analysis..... 76**
- 21) **Percentage of farmed (black) and native (white) juvenile Atlantic salmon in collections from the Magaguadavic River, New Brunswick, 1996..... 77**
- 22) **Percentage of farmed (black) and native (white) juvenile Atlantic salmon in collections from the Magaguadavic River, New Brunswick, 1997..... 78**
- 23) **Percentage of farmed (black) and native (white) juvenile Atlantic salmon in collections from the Magaguadavic River, New Brunswick, 1998..... 79**
- 24) **Distribution of Known native (cross-hatched) farmed (black) and unknown origin (white) juvenile Atlantic salmon from the Digdequash River (1998); as determined by a discriminant function analysis..... 80**

- 25) **Distribution of Known native (cross-hatched) farmed (black) and unknown origin (white) juvenile Atlantic salmon from the Waweig River (1998); as determined by a discriminant function analysis..... 81**
- 26) **Temperature profile for the Kedron Stream, Magaguadavic River, New Brunswick in 1998. The temperature recorder's maximum temperature (21.5 degrees celcius) was reached from late July to mid-September resulting in a plateaued reading..... 82**
- 27) **Temperature profile from the Digdequash River, New Brunswick, 1998... 83**
- 28) **Temperature profile from the Waweig River, New Brunswick, 1998..... 84**
- 29) **Temperature profile from the Linton Stream of the Magaguadavic River, New Brunswick in 1998..... 85**
- 30) **Scale from a 1-year-old Atlantic salmon collected 15 September 1998 from the Kedron Stream of the Magaguadavic River N. B. This fish was predicted farmed origin by the discriminant function analysis. Because this fish was captured approximately 40 km from a hatchery and up a side stream, it is more likely that it was an improperly marked stocked fingerling from stocking that occurred in the Kedron Stream in 1997..... 86**

### **Abstract**

A procedure of classification using a discriminant function analysis was developed to determine the farmed or native natal origin of Atlantic salmon juveniles in the Magaguadavic River, New Brunswick. Farmed juveniles enter this river as escapees from three commercial aquaculture hatcheries. The procedure evaluated measured scale characteristics from the first year of growth, of farmed and native juveniles of known origin, for their power as predictors of derivation. Eight scale characteristics proved to be significant predictors of origin. In a jackknife cross-validation, the function developed from the characteristics proved to be 90.3% accurate in predicting the origin of juvenile Atlantic salmon in the Magaguadavic River. The procedure was then applied to unknown origin juveniles sampled from the Magaguadavic, Waweig and Digdequash rivers in New Brunswick. All of these rivers support hatcheries. Juvenile salmon sampled in the Magaguadavic River in 1996, 1997 and 1998, were determined to be 34%, 63% and 42% of farmed origin, respectively. During 1998, 9% of the juveniles from the Digdequash River were of farmed origin, and 42% of the juveniles in the Waweig River were of farmed origin. The study indicated that substantial numbers of farmed juveniles escaped from hatcheries and occupied juvenile salmon habitat in all three rivers.

## **Acknowledgements**

I would like to thank Fred Whorisky, Jon Carr and the rest of the Atlantic Salmon Federation for their financial, intellectual and technical support. Derrick Knox provided technical expertise which proved invaluable both in the field and in helping me gain an understanding of commercial fish culture. Dan Toews and Marty Snider provided valuable guidance throughout and I thank them for their assistance.

I would like to thank my mother for her support and encouragement. The guidance of my brother, Kevin, has been invaluable. His enthusiasm continues to inspire me. He remains one of the few people I know for whom biology is a burning passion.

I would like to thank my co-supervisors Gilles Lacroix and Michael Dadswell. They taught me that the more people try to suppress scientific research, the more important it is that that research be done.

Finally, my deepest thanks go to my wife, Lisa. She has at times filled the role of statistician, coach, psychologist, proofreader and devil's advocate. It is invaluable to have a partner who can challenge you scientifically, relieve you stress with supportive words and who plays a mean game of shuffleboard on "Wing Night".

## **Dedication**

I would like to dedicate this to my father, James L. Stokesbury.

## **Introduction**

Commercial farming of salmon has become a growth industry over the last 30 years. For example, in the Maritime Provinces of Canada production of farmed salmon has grown from 6 t in 1979 (Carr et al. 1997a) to 19 700 t in 1997 (Chang 1998). Further, the farmed salmon industry in 1997 included over 90 marine growout sites and 31 freshwater hatcheries producing approximately 8 600 000 smolts annually (Chang 1998). A large proportion of salmon farming in the Maritime Provinces is centered in southwestern New Brunswick. This is located in close proximity to the Cobscook, Maine salmon farming industry which produced 12 140 t of farmed salmon in 1997 (Baum 1998).

Each year a percentage of adult farmed salmon escape into the wild (Gausen and Moen 1991; Lund et al. 1991; Laura and Saegrov 1991; Webb et al. 1991; Webb and Youngson 1992; Webb et al. 1993, Hansen et al. 1993; Skaala et al 1996; Hansen et al. 1997; Carr et al. 1997a). Estimates of the percentage of adult salmon that escape annually from sea cages vary from 1-2% in British Columbia (Alverson and Ruggerone 1997) to 1.6% in Norway (Bailey 1998) to less than 2% in New Brunswick (Chang 1998). Escape of farmed salmon (see Appendix 1 for glossary of terms) into the wild also occurs from fresh water hatcheries and has been reported in New Brunswick (Stokesbury and Lacroix 1997; Whoriskey et al. 1998) and Ireland (Clifford et al. 1998a) but has yet to be quantified.

The escape of farmed salmon into the wild is of concern because farmed salmon may differ both genetically (Allendorf and Phelps 1980; Cross and King 1983; Friars et al. 1990; Gjedrem et al. 1991; Youngson et al. 1991; Fleming and Gross 1992; Friars et al. 1995; Fleming et al. 1996; Jonasson 1996; Einum and Fleming 1997; Peterson and Jarvi 1997) and behaviorally (Berejikian 1995; Berejikian et al. 1996; Einum and Fleming 1997) from their native counterparts. Their presence in the wild may, therefore, have ecological (Hvidsten and Lund 1988; Johnsen and Jensen 1991; Hastein and Lindstad 1991; Einum and Fleming 1997; McGinnity et al. 1997) and genetic (Verspoor and Hammar 1991; McGinnity et al. 1997; Clifford et al. 1998b; Lacroix et al. 1998) impacts on native stocks.

#### *Effects of cultured rearing on salmonids*

*Environmental Impacts.* Raising Atlantic salmon in a controlled environment causes selection for traits that are different than those selected for in the wild environment (Gross 1998). The environmental effects of cultured rearing on salmonids include acceptance of high density rearing (Ewing and Ewing 1995), increased aggressive behaviour compared to native counterparts (Berejikian et al. 1996; Einum and Fleming 1997), lack of predator avoidance (Berejikian 1995; Johnsson et al. 1996), morphological adaptations to culture (Friars et al. 1990; Fleming et al. 1994; Peterson and Jarvi 1997), and high growth rate (Fleming and Einum 1997). These traits may be beneficial to fish while in culture, but they are most likely detrimental to fish in the wild.

The release of cultured fish into areas occupied by native stocks may have several negative ecological impacts on the native population. For example, elevated



densities may increase competition for resources (Einum and Fleming 1997; McGinnity et al. 1997) and may lead to reduced prey densities. Other negative impacts may include attraction of predators (Hvidsten and Lund 1988) and introduction of disease to native populations (Johnsen and Jensen 1991; Hastein and Lindstad 1991). Therefore, farmed salmon introductions threaten native salmon ecologically, as well as genetically.

*Genetic Impacts.* The genetic distinction between farmed and native salmon has become so pronounced that it has been suggested that farmed salmon should be recognized as a new biological entity, *Salmo domesticus* (Gross 1998). This genetic distinction is present for three broad reasons. First, farmed salmon are frequently imported from other stocks or countries because non-indigenous strains may have superior growth patterns (Youngson et al. 1991). Second, small numbers of non-indigenous salmon are often used as founding fish to establish farmed populations (Allendorf and Phelps 1980). This may result in random genetic change (Mayr 1942; Mac Arthur and Wilson 1967; Allendorf and Phelps 1980). Third, selective breeding and rearing in controlled environments experienced by farmed salmon may result in an evolutionary lineage distinct from native stocks (Gross 1998). This occurs as existing genetic differences between farmed and native salmonids are exacerbated by selective breeding practices used in the aquaculture industry (Cross and King 1983; Friars et al. 1990; Skaala et al. 1990; Fevolden et al. 1991; Thorpe 1991; Fevolden et al. 1993; Fjalestad et al. 1993; Friars et al. 1995; Hesthagen et al. 1995; Jonasson 1996).

There are many benefits that accrue to the aquaculture industry from the selective breeding of salmon. Intentional selection may reduce production costs, shorten production time, improve flesh quality, increase domestication (tameness and low stress) (Gjedrem et al. 1991) increase growth (Friars et al. 1990; Friars et al. 1995; Einum and Fleming 1997) and disease resistance (Beacham and Evelyn 1992; Fjalestad et al. 1993; Fjalestad et al. 1996). Hence, it is important for the industry to keep salmon in culture over generations so that they may be selectively bred to increase profit margins.

Cultured salmon may also have genetic traits that are selected unintentionally. These effects may include: a lower maturation rate and less spawning success compared to native counterparts (Fleming and Gross 1992; Fleming et al. 1996; Petersson and Jarvi 1997) phenotypic differences (Fleming and Einum 1997) and changes in body composition (Riddell et al. 1981). Seasonal run timing of smolts (Orciari and Leonard 1996) and adults (Hansen and Jonsson 1991) growth capacity (Conover 1990; Nicieza et al. 1994) homing to native rivers and streams (Bams 1976; McIsaac and Quinn 1988), and habitat preference (Hesthagen et al. 1995) may also be affected by unintentional selection in culture. These traits are generally not selected for deliberately. Regardless, they contribute to the genetic differentiation between farmed and native salmon.

The implications of genetic differences between farmed and native salmon are significant to the health of the native stocks. That is, each population of native Atlantic salmon has become genetically adapted to its specific river for increased productivity and survival (Saunders 1991; Hindar et al. 1991). However, farmed

escapees breed successfully with native salmon in rivers (Gausen and Moen 1991; Lura and Saegrov 1991; Lund et al. 1991; Crozier 1993; Clifford et al. 1998b). This inter-breeding is the pathway for genetic mixing between the two groups through introgression<sup>1</sup> (Verspoor and Hammar 1991). Introgression could cause an overall genetic shift in the native stocks away from naturally selected traits (Riddell and Leggett 1981) which may reduce the overall fitness of native populations (Verspoor and Hammar 1991; McGinnity et al. 1997; Lacroix et al. 1998).

#### *Identification of farmed or native origin*

Escapees enter the wild during their juvenile and adult life stages. Because of the potential negative genetic and ecological impacts of escapees on native salmon stocks, it is important for resource managers to be able to determine the number of escapees entering the wild. To do this, both adult and juvenile escapees must be identified when captured in the wild.

Procedures have been developed to identify both juvenile and adult salmon. The procedures have included morphological identification, genetic identification, and identification through scale analysis. These methods vary in appropriateness according to the age of the fish.

#### *Adult Identification*

Most studies that identified the farmed or native origin of salmonids have concentrated on identifying adult escapees from marine grow-out sites (Lund et al. 1991; Ikonen et al. 1991; Crozier 1991; Hansen et al. 1993; Bernard and Myers 1996; Koljonen and Pella 1997; Crozier 1998). Typically, the methods used have been

---

<sup>1</sup> Introgression is the introduction of novel genetic traits into a population through inter-breeding and backcrossing (Hindar et al 1991).

based on the physical characteristics of the fish. Often, the extensive time that adult fish spend in culture alters their morphology and condition and makes them relatively easy to distinguish from their native counterparts (Fleming et al. 1994).

In several studies, scale characteristics have been used to distinguish farmed and native adult salmon. For example, Lund and Hansen (1991) developed a procedure for differentiating between farmed and native salmon using six characteristics (e.g., smolt size, smolt age, transition from fresh water to salt water, sea winter bands, summer checks, replacement scales). However, salmon that escape as smolts or earlier are difficult to detect using this procedure (Lund and Hansen 1991).

Morphological characteristics of farmed salmon have also been used to identify the farmed or native origin of adult salmon. These include characteristics such as abnormalities of the snout and opercula (Crozier 1991; Crozier 1998), fin erosion (Crozier 1991; Lacroix et al. 1997; Crozier 1998), head proportion, and depth of caudal peduncle (Fleming et al. 1994). However, in contrast to adults, the limited time that the juvenile escapees spend in culture makes them more difficult to discern from native juveniles by external examination (Fleming et al. 1994).

#### *Juvenile identification*

Several researchers have attempted to develop procedures for distinguishing farmed juvenile salmonids from those of native origin. Six of these studies were effective. First, Zhang et al. (1995) correctly identified the origin of 89% of juvenile chinook salmon, *Oncorhynchus tshawytscha* (Walbaum, 1792), using daily growth increments on otoliths. Similarly, Korman et al. (1997) correctly categorized the

origin of 98% of juvenile chinook salmon using otoliths and length and weight data. Unfortunately, otolith examination necessitates the lethal sampling of fish. Given that some Atlantic salmon populations are dangerously low at present (Amiro 1998), lethal sampling of salmon is seldom an option for researchers.

The procedure of Clifford et al. (1998a) may be the most effective for identifying juvenile salmon of farmed origin in the wild. Their procedure uses mitochondrial and minisatellite DNA analysis to determine the parentage of salmon part. However, there are shortcomings to this procedure. First, the cost of performing a substantial amount of DNA analysis may be limiting. Second, the results may be confounded by past escapees that may have interbred with native stocks in the river. For example, Clifford et al. (1998a) reported that some of the juveniles that tested positive for the *Ava* II-B RFLP (a genetic marker for the cultured stock) were probably born in the wild. They suggested that because of where they were captured (20-300 m upstream of the hatchery) and the size of the fish that were age 0+ in July, the fish were probably not direct juvenile escapees. These fish were possibly the offspring of past escapees that had bred in the river. Therefore, DNA analysis on juveniles from rivers that have been occupied by adult escapees can give confounding results.

Three studies successfully distinguished between farmed and native origin salmon using scale characteristics only. First, Unwin and Lucas (1993) used a linear discriminant function analysis based on the location of the first annulus (distance from focus) and the fork length and age of chinook salmon. Similarly, Stokesbury and Lacroix (1997) determined natal origin of juvenile Atlantic salmon using a

discriminant function based on the number of circuli in the first annual zone and the back-calculated length at age one.

Marcogliese and Casselman (1998) developed a procedure to classify rainbow trout (*Oncorhynchus mykiss*) from farmed or native origin. This procedure used the ratio between circuli spacing and circuli thickness on either side of the first two checks on the scale. This procedure had a mean error rate of only 8.9%. The analysis was developed to bypass the subjectivity of assigning a first annulus. Instead, the first two checks were identified and subjectively assigned a rating between 1 and 9. This procedure was effective when applied to juvenile rainbow trout.

Although Unwin and Lucas (1993), Stokesbury and Lacroix (1997), and Marcogliese and Casselman (1998) were able to identify farmed origin fish after their first year, they could not accurately identify fish that entered the wild *during* their first year. This is problematic as approximately 95% of juveniles contained in hatcheries are age 0+ (Chang 1998) and, therefore, juveniles usually escape at age 0+. Thus, there is a need for accurate identification of 0+ escapees.

As noted, the procedures of Unwin and Lucas (1993) and Stokesbury and Lacroix (1997) were ineffective when applied to fish that are in their first year of life. That is, the discriminant function procedures require the presence of the first annulus to establish the criteria for the function. At age 0, the first annulus of the fish has not yet formed. Therefore, these procedures may not be used when evaluating age 0+ escapees. Further, the procedure of Marcogliese and Casselman (1998) may not be appropriate for use on age 0+ juvenile Atlantic salmon for another reason. Their

procedure required the existence of two checks. However, two checks are often not present on the scales of age 0+ Atlantic salmon.

The purpose of the present study was twofold. The first purpose was to develop an identification procedure capable of determining the origin of Atlantic salmon at age 0+. The second purpose was to determine the proportion of farmed origin juvenile Atlantic salmon present at sampling sites in the Magaguadavic (1996-1998), Digdequash (1998), and Waweig (1998) rivers. This was done by discriminant function analysis derived from scale characteristics from the first year of growth. A procedure such as this was needed to determine the contribution to the “wild” population, of juvenile escapees from commercial salmon aquaculture hatcheries along the study rivers.

### **Site selection**

The Magaguadavic River in southwestern New Brunswick was chosen as the focus for this study (Figure 1). The Magaguadavic River drains into Passamaquoddy Bay, in the Bay of Fundy. It is the sixth largest river in New Brunswick and drains an area of 1812-km<sup>2</sup>. The river flows 97 km from its beginning at Magaguadavic Lake to the head of the tide. There are 9300 km<sup>2</sup> of accessible fish habitat, 4600 km<sup>2</sup> of which is suitable rearing habitat for anadromous salmon (Carr et al. 1997a).

St. George, New Brunswick is located at the head of the tide of the Magaguadavic River. In 1934, a concrete dam was constructed on the Magaguadavic River at St. George (to replace a log dam) that is still maintained. A small fishway operates at the St. George dam and has been operated by the Atlantic Salmon Federation since 1992 (Carr et al. 1997a). This is the only passage to the Magaguadavic River from the ocean.

The Magaguadavic River provides an exceptional site to study farmed and native Atlantic salmon. It had a native run of salmon that consisted of 800-900 adults in the 1980's. However, by 1999 the run dropped to approximately 30 fish (Whoriskey 1999). Also, the mouth of the river is close (within 10 km) to 70% of Canada's East Coast commercial salmon aquaculture grow out sites (Carr et al. 1997a). The river also supports three commercial aquaculture hatcheries, which produce in excess of 2 million smolts annually that are used in commercial aquaculture (Stokesbury and Lacroix 1997).



There is a database of farmed and native adult salmon entering the Magaguadavic River from 1992 to the present (Carr et al. 1997a). Adult escapees have entered the river every year since 1992 (Carr 1995; Carr et al. 1997a; Carr et al. 1997b; Lacroix et al. 1997). Analysis of carotenoid pigments of eggs in redds in 1993 showed that farmed salmon spawned successfully in the river (Carr et al. 1997b). The Magaguadavic River has also been stocked by the Department of Fisheries and Oceans (1987, 14 644 parr; 1988, 2 034 smolts; 1989, 10 771 smolts; Carr et al. 1997a) and by the Atlantic Salmon Federation (1997, 2 267 juveniles) with cultured fish that were the progeny of native Magaguadavic River salmon.

Through the use of a discriminant function analysis on scale characteristics, Stokesbury and Lacroix (1997) determined that between 51.0% and 67.2% of the smolts migrating from the river were juvenile escapees from one or more of the three hatcheries operating in the system. Therefore, the Magaguadavic River provided a wild study site where juvenile escapees and native salmon were both present.

Three other rivers/streams were chosen for this study that are close to the Magaguadavic River (Figure 1). The Dennis Stream, the smallest of the rivers, was used to gain native controls. There was no hatchery on the Dennis Stream and it had not been stocked in recent years (R. Jones, Department of Fisheries and Oceans, personal communication). Therefore, all juvenile Atlantic salmon in the Dennis Stream are presumably of native origin. The Waweig and Digdequash Rivers did support commercial salmon aquaculture hatcheries. Juvenile Atlantic salmon escapees had not been reported from these rivers. However, 24 juvenile coho salmon (*Oncorhynchus kisutch*) were found in the Digdequash River system in 1976. They

were believed to be the progeny of aquaculture escapee coho salmon from Maine, or stocked coho from New Hampshire or Massachusetts (Symons and Martin, 1978).

Sampling sites in the Magaguadavic River were chosen for accessibility and distance from, or closeness to, commercial hatcheries. The sites were also chosen to correspond with historical sampling completed by the Atlantic Salmon Federation. The sampling site used in the Digdequash River was chosen as it was approximately 30 km upstream from the only commercial hatchery in the system. The Waweig River sampling site was chosen as it was directly (approximately 0.25 km) downstream from the only commercial hatchery in the system.

## Materials and Methods

A linear discriminant function was developed and tested that predicted the farmed or native origin of unknown origin Atlantic salmon juveniles. Discriminant function analysis, using scale characteristics as independent variables, has been used previously to classify adult salmon to approximate cline of origin (Lear and Misra 1978). Discriminant function analysis has also been used to classify the farmed or native origin of adult (Ikonen et al. 1991; Hiilivirta et al. 1998) and juvenile (Stokesbury and Lacroix 1997) salmon (Table 1). To date, no method has accurately classified age 0+ salmonids to farmed or native origin.

The discriminant function was developed from two groups of “known origin” Atlantic salmon parr. Unknown origin juveniles were sampled in the Magaguadavic River, Digdequash River and Waweig River. These three rivers supported commercial salmon aquaculture hatcheries.

### *Samples*

The Magaguadavic River contained three groups of juvenile salmon. The groups were juvenile escapees, native origin and stocked origin. The native origin group may have included native salmon whose parents were of farmed origin. However, genetic screening was outside of the scope of this study. Therefore, these salmon were classified as native in origin for the purpose of this study.

*Stocked Fish.* The Atlantic Salmon Federation stocked juvenile salmon in the Magaguadavic River (N = 2 767), system in 1997. These juveniles were adipose fin clipped prior to release. They were the f1 progeny of native broodstock. The

broodstock were taken from the fall run of returning native adults to the Magaguadavic River in 1996 (Carr and Whoriskey 1998). Stocked salmon were not representative of either of the two populations, juvenile escapee or native origin, which were to be evaluated in the discriminant function analysis. Therefore, salmon captured that had clipped adipose fins were removed from the data set.

*Known origin farmed.* Cultured juveniles were sampled in 1997 at two commercial aquaculture hatcheries operating on the Magaguadavic River system (Figure 2). Thirty-two Atlantic salmon parr were sampled at the Connors Brothers hatchery and 35 Atlantic salmon parr were sampled at the Stolt Sea Farm hatchery (Table 2). The hatchery fish were all sampled 12 months after hatching, therefore, they were age 1+.

*Known origin native.* The known origin native sample consisted partly of 30 Atlantic salmon parr sampled from side streams along the middle reaches of the Magaguadavic River in 1996 (Figure 3). These parr were captured by electrofishing from sites in the Kedron Stream, lower Trout Brook and Pischahagen Stream (Table 2). These sites were selected because they were greater than 25 km from any hatchery. They were also located in side-streams which had not been recently stocked and which were greater than 0.5 km from the main river. In addition, all three areas contained spawning grounds for native Atlantic salmon that were in use in 1996 (Carr et al. 1997a).

Autumnal downstream migration of parr has been reported (Riddell and Leggett 1981; Youngson et al. 1983; Cunjak et al. 1989) so some downstream movement of juvenile escapees from their site of escape may be expected. However,

because of the distance of the sampling sites from hatcheries and the presence of native spawning adults in the areas, it was assumed that the sampled juveniles were of native origin.

The remainder of the known origin native sample ( $n = 27$ ) was taken from the Dennis Stream on 23 July 1998 (Table 2). The Dennis Stream had not been recently (past 10 years) stocked. Also, there was no hatchery in the Dennis Stream system. Dennis Stream juvenile salmon samples were assumed to be of native origin.

*Applicability to Digdequash and Waweig unknown origin juveniles.* In order to use this procedure on juvenile salmon of unknown origin from the Digdequash and Waweig Rivers, the similarity of smolt age in the region was evaluated. There is a large range in ages of smoltification of Atlantic salmon (Hutchings and Jones 1998) that corresponds roughly to latitude. Smolting ages range from 1 year-old smolt in France (Bagliniere and Maisse 1985) to 10 year-old smolt in northern Quebec (Power 1969).

The Digdequash and Waweig Rivers are located between the Dennis Stream and Magaguadavic River (Figure 1). Comparison of age of smolts produced by these rivers cannot be made due to lack of data. However, data are available for two rivers at greater distances from the Magaguadavic River: the Narraguagus River, Maine (mean smolt age = 2.2 years; Baum 1997) to the southwest and the Saint John River, New Brunswick (mean smolt age = 2.5 years; Marshall and Jones 1996) to the northeast (Figure 1). These rivers produce smolts of similar age to the Magaguadavic River (mean smolt age = 2.2 years; Martin 1984). It was assumed that the age of the smolts produced in the Digdequash and Waweig Rivers, with the assumption of

similar growth conditions, were similar to those of the Magaguadavic River. Similar growth rate and, hence, smolt age allow the evaluation of the farmed or native origin of juveniles in these rivers using criteria developed using juveniles from Magaguadavic River and Dennis Stream.

*Unknown Origin juveniles.* Atlantic salmon juveniles were sampled in the Magaguadavic River in 1996 (n = 123), 1997 (n = 143) and 1998 (n = 127) (Table 2). Sampling periods were 4 to 12 September in 1996 and 9 to 10 September 1997. Juveniles were collected from 13 sites in 1996 (Figure 4) and 6 sites in 1997 (Figure 5). Juveniles sampled in the wild in 1998 were captured during five periods, approximately monthly, from June to October. Sampling during 1998 was performed at three sites in the Magaguadavic River (Figure 6).

Unknown origin Atlantic salmon juveniles were collected, approximately monthly from June to October 1998, in the Digdequash River (n = 87) and Waweig River (n = 97) (Table 2). The collections in the Digdequash River were performed approximately 30 kilometers upriver of the river's only hatchery. This site was chosen as it was likely to provide mostly native origin juveniles. The Waweig River collections were performed approximately 0.25 kilometers downstream of the river's only hatchery. This site was chosen as it was likely to provide mostly juvenile escapees. Therefore, as escapees would be expected to be found below hatcheries and not far upstream of hatcheries, it was possible that these sampling sites could provide indirect corroboration that the procedure was valid.

### *Sampling Procedure*

Electro-fishing gear was used to capture the juvenile salmon in the wild. A dip net was used to collect the hatchery salmon. In all cases salmon, once captured, were handled using the same procedure. The salmon were removed from the river or tank. They were then anesthetized using a clove oil solution (Soto and Burhanuddin 1995). The fish were then weighed, measured (fork length), and visually checked for fin clips. Scale samples were taken from an area posterior to the dorsal fin and above the lateral line. The scales were stored on acetate slides as described in Power (1964). The fish were allowed to recover in a bucket of fresh water. They were then re-distributed over the sampling site in the wild or returned to the tank of origin in the hatchery.

### *Temperature and pH profiles*

Water temperature has an effect on the growth pattern of juvenile salmonids (Brett et al. 1969; Lagler et al. 1977; Riddle and Leggett 1981; Conover 1990). Therefore, to assess the growth conditions in streams from which juveniles were sampled, water temperature and pH were monitored. Water temperature in the Magaguadavic River was sampled using VEMCO™ temperature recorders deployed at two sites (the Kedron Stream and the Linton Stream). The Kedron Stream recorder was deployed on 8 July 1998 and collected on 27 October 1998. The Linton Stream recorder was deployed on 24 June 1998 and collected on 27 October 1998. Temperature measurements were also recorded at the Tomaston Corner site on the Magaguadavic River, monthly from June to October 1998.

On 20 May 1998, temperature recorders were deployed in the Digdequash and Waweig Rivers. They were collected on 16 November 1998. All temperature recorders collected temperature data at hourly intervals.

Water samples were collected from the five sites mentioned above on a monthly basis from July to October 1998. Measurements of pH were later taken from the samples using an Accumet™ 910 pH meter with a gel-filled combination electrode (reference element silver chloride; electrolyte = 4 M potassium chloride saturated with silver chloride).

### *Scale Analysis*

Scales that were stored on acetate slides were evaluated using an image analysis system. This consisted of a microscope, video camera, frame grabber and laptop computer. The images were captured as JPEG™ images and transferred to a desktop computer. The images were then evaluated using Image Tool™ software.

Images of scales were analyzed on a desktop computer screen. The scale was magnified (210 X) in the image. Linear measurements were taken in the optical units of Image Tool™ (1 optical unit = 0.678  $\mu\text{m}$ ). Area measurements were taken in optical units squared.

All but two scale measurements were linear and taken along a line perpendicular to a reference line (Figure 7) as described in Schwartzberg and Fryer (1993). The distance was measured (optical units) between each successive pair of circuli for the first six pairs (Figure 8) allowing evaluation of wild parr at this latitude (approximately 45° 25' N) by the fall of their first year of life (Lear and Misra 1978).



This also allowed assessment of scale characteristics that had been established by commercial hatchery fish before their first grading (D. Knox, D. F. O., St Andrews Biological Station, N. B., unpublished data).

Two of the measurements taken were non-linear. These were aging, which was done by counting successive annuli. Other measurements were the area of the focus (inside of the first circuli) which was measured by tracing the inside of the first circuli, and using the “area” measurement capability of Image Tool™.

Preliminary statistics performed on the scale measurements included conversion of optical units to  $\mu\text{m}$ :

$$(1 \text{ optical unit} / 1.475 = 1 \mu\text{m} )$$

The mean and standard deviation of the distances of the first six circuli were also calculated.

### *Statistics*

*Univariate and multivariate outliers.* The data were checked for univariate outliers (i.e. scores  $> \pm 3$  standard deviations from the mean and discontinuous from the distribution). The data were also checked for multivariate outliers using Mahalanobis' distance ( $D_{ab}^2$ ):

$$D_{ab}^2 = (n - g) \sum \sum w_{ij} \bullet (X_{ia} - X_{ib}) (X_{ja} - X_{jb})$$

where,  $p$  is the number of variables,  $w_{ij}$  is an element of the within-groups covariation matrix, and  $X_{ia}$  is the mean of the  $i$ th variable in group  $a$ .

Five univariate outliers were found by examination of histograms of standardized scores. The univariate outliers were recoded so that the scores remained extreme (2 standard deviations from the mean but within the distribution of the data

(Tabachnick and Fidell 1989). Using Mahalanobis' distance, seven cases were multivariate outliers ( $p < .001$  d.f. = 9). The discriminant function analysis was run with these cases both included and excluded. When compared, no difference was apparent in the results of the two trials. Therefore, the analysis was performed with all cases included.

*Logistic regression.* In order to establish the validity of combining the “known origin native” groups from the Magaguadavic River and the Dennis Stream, a logistic regression was conducted. This was done to establish whether these fish were from similar populations and could be legitimately combined as one group. This tested for significant differences in the measured variables between the groups. Since the number of subjects (56) was small as compared to the number of predictors being tested for (9), the significance for the logistic regression was set at  $p < 0.01$ . None of the variables tested were significantly different between the two groups (Table 3). Therefore, the two groups were combined to form the “known origin native” group used to develop the discriminant function.

*Discriminant function analysis.* A discriminant function analysis was conducted using nine scale measurement variables as predictors of membership in two groups. That is, given a significant difference on one or more variables between groups, you can predict which group a subject came from. Therefore, the independent variables are the predictors and the dependent variables are the groups (Tabachnick and Fidell 1989).

Discriminant function analysis has no direct calculation of error. Instead the relevance of classification is derived from jackknife cross-validation, Wilk's Lambda,

and canonical correlation. Cross-validation tests the accuracy of classification of the function by evaluating the subjects that were used to form the function (i.e. the known groups). In cross-validation, bias is created as the subjects are classified by a function that data from that subject in part, calculated. However in jackknife cross-validation, bias is reduced by removing the data from a case when the coefficients used to assign it to a group are calculated (Tabachnick and Fidell 1989). Therefore, a case is not classified by a function it was involved in calculating. Wilk's Lambda evaluates the reliability of a set of predictor variable by giving an F value. Canonical correlation indicates the proportion of variance shared between groups and predictors in a function (Tabachnick and Fidell 1989).

There are several limitations to discriminant function analysis. Lack of robustness due to small sample sizes can be problematic. To be robust the sample size should produce 20 degrees of freedom. Therefore, the smallest sample size should include at least 20 cases (Tabachnick and Fidell 1989). Two other limitations to discriminant function analysis are; first, that discriminant function analysis cannot predict membership if groups are formed randomly (Tabachnick and Fidell 1989). Secondly, discriminant function analysis is sensitive to the inclusion of outliers (Tabachnick and Fidell 1989). Therefore, the data must be screened for univariate and multivariate outliers and tested for their effect on the data set.

#### *Choice of independent variables (predictors)*

In order to develop the discriminant function, independent variables that might be predictors of the farmed or native origin of juvenile salmon had to be investigated. These predictors were measured, or derived from measurements of,

scale samples from the two known origin groups. First, the area of the focus was measured. This is the nuclear, central area of the scale (Lagler et al. 1977) inside the first circuli (Cailliet et al. 1986). The focus is the first part of the scale to form and originates as platelets in the dermis (Lagler et al. 1977). Focus formation can occur in native Atlantic salmon of 25 mm (G. L. Lacroix, Fisheries and Oceans Canada, St. Andrews, New Brunswick, Canada, unpublished data) and farmed salmon of 28 mm (R. H. Peterson, Fisheries and Oceans Canada, St. Andrews, New Brunswick, Canada, personal communication). Because of the size difference of the two groups of fish at platelet formation, it was predicted that the size of the focus would be proportionally bigger in farmed origin fish than in native origin fish.

Second, the distance between the consecutive circuli (bony rings concentric around the focus of the scale; Cailliet et al. 1986) were measured. Circuli form regularly. When growth is accelerated, circuli form farther apart. When growth is slowed circuli form closer together (de Vries and Frie 1996). Therefore, accelerated growth rates in hatcheries should result in larger spacing of circuli for farmed fish than for native fish.

Approximately twelve circuli form in the first year of life in juvenile Atlantic salmon at the latitude (approximately 45° 25' N) of the Magaguadavic River (Lear and Misra 1978). Therefore, it was decided to investigate growth increments within the first six circuli pairs to allow assessment of origin in the fall of the first year of juvenile growth.

Circuli spacing has been used successfully by Marcogliese and Casselman (1998) to separate farmed and native origin rainbow trout. Farmed and native

Atlantic salmon are subject to growth conditions similar to those of rainbow trout. Therefore, it was predicted that circuli spacing would also be effective in distinguishing between farmed and native origin juvenile Atlantic salmon.

The mean and standard deviation of distance between the first six circuli were also evaluated for their predictive power. As growth is accelerated in hatcheries, it was predicted that the mean distance of the first six circuli pairs would be greater in the farmed origin fish than in native origin fish. Further, because growth in hatcheries is controlled, it was predicted that the standard deviation of the first six circuli of farmed fish would be smaller than for the native origin sample.

*Dependent variables (Groups)*

The groups were farmed origin and native origin, juvenile Atlantic salmon. The function was derived from two “known origin” groups of juveniles: one from the Magaguadavic River and Dennis Stream (known origin native), one from the Connors Brothers and Stolt Sea Farms hatcheries (known origin farmed). We used the linear discriminant equation (D):

$$D = B_0 = B_1X_1 + B_2X_2$$

The X's are the values of the independent variables and B's are coefficients estimated from the data.

Using the discriminant scores, cases were classified in the two groups using Bayes' rule (P (G<sub>i</sub>/D)):

$$P (G_i/D) = \frac{P (D/G_i) P (G_i)}{\sum P(D/G_i) P (G_i)}$$

In Bayes' rule the P(G<sub>i</sub>) represents prior probability and D represents the discriminant score.

The classification of the “known origin” juveniles into farmed or native groups provides a measure of the accuracy of the function. This was tested in a jackknife cross-validation (Tabachnick and Fidell, 1989). The discriminant function analyses were run on the “unknown origin” juveniles collected from the Magaguadavic (1996-1998), Digdequash (1998) and Waweig Rivers (1998), using the variables that were significant ( $p < 0.05$ ) predictors of origin in the development of the function. Farmed or native origin was predicted for these samples.

Of the original 124 cases, none were dropped from the analysis because of missing data. Investigation of the correlation matrix revealed no evidence of multicollinearity or singularity (all correlations were less than  $r = 0.70$ ).

## Results

### *Discriminant function for Predicted Origin.*

One discriminant function was calculated with  $X^2(8) = 137.68$ ,  $p < .001$  using the following 9 variables: the distance between each of the circuli pairs 1-6; area of the focus; mean distance between the first six circuli; standard deviation of the distances between the first six circuli. The discriminant function accounted for 100% of the between group variability. Correlation between the predictors and the discriminant function suggested that the best predictors for distinguishing between farmed and native origin Atlantic salmon juveniles were the mean of the first six circuli; circuli pair two; the area of the focus; circuli pair three; circuli pair four; standard deviation of the first six circuli; circuli pair five and circuli pair one (Table 4). Circuli pair six did not load significantly and was dropped from the analysis.

Farmed origin salmon had a larger focal area (Figures 9, 10), larger distance between the first five consecutive circuli pairs (Figures 11, 12) and a larger mean distance for the first five circuli pairs (Figure 13) than did the native origin group (Figures 14, 15). However, the native origin group had a larger standard deviation for mean distance between the first six circuli pairs (Figures 16, 17, 18) than did the hatchery origin salmon (Figures 19). With the use of the jackknife cross-validation procedure for the total usable sample of 124 salmon, 118 (90.3%) were classified correctly, compared to 62 (50.0%) that would be correctly classified by chance alone (Table 5).

*Application of Discriminant function to wild juveniles from the Magaguadavic River.* The eight variables that predicted origin significantly in the development of the discriminant function were used to determine the origin of juveniles from the Magaguadavic River “unknown origin” group. The discriminant function analysis predicted that of the “unknown origin” wild juveniles from the Magaguadavic River in 1996-1998, (47.3%) were of farmed origin and (52.7%) were of native origin (Figure 20, Table 5). In individual years, it predicted that in the Magaguadavic River 34% of the juveniles were of farmed origin and 66% were of native origin in 1996; 63% were of farmed origin and 37% were of native origin in 1997; 42% were of farmed origin and 58% were of native origin in 1998 (Table 5).

Over the three years, 81% of the predicted juvenile escapees were located within 5 km of a hatchery instillation (Figures 21, 22, 23). However, a correlation analysis indicated no significant correlation between the proportion of farmed escapees in samples and the distance to the closest hatchery ( $f = .267$ ).

*Application of Discriminant function to wild juveniles from the Digdequash River.* The discriminate function analysis developed on the Magaguadavic River juveniles was applied to the “unknown origin” juveniles that were collected from the Digdequash River in 1998. Previous measurements of accuracy including the jackknife cross-validation were considered to apply. The analysis of juveniles from this site predicted that only 6 (8.8%) of the 87 juveniles were of farmed origin and 81 (91.2%) were of native origin (Figure 24, Table 5). This is a difference of 38.5% in farmed escapees compared to the Magaguadavic River.



*Application of Discriminate function to wild juveniles from the Waweig River.*

The discriminant function was applied to predict group origin of the Waweig River “unknown origin” juvenile salmon sample. Of these, 41 (42.3%) of 97 juveniles were predicted to be of farmed origin and 56 (57.7%) were predicted to be of native origin (Figure 25; Table 5). This was similar to the level of hatchery escapees identified in the Magaguadavic River in 1998.

*Temperature and pH*

The temperature and pH profiles of the Magaguadavic, Digdequash and Waweig Rivers and the Dennis Stream were similar. However, the Linton Stream profiles differed from the other sites. Temperature profiles from the Kedron Stream (mean = 16.3 °C; S.D. = 5.86; Figure 26), the Digdequash River (mean = 16.2 °C; S.D. = 6.04; Figure 27) and the Waweig River (mean = 15.2 °C; S.D. = 5.91; Figure 28) were similar. The mean temperature recorded by the Kedron Stream recorder may be lower than actually occurred as the recorder reached it's maximum temperature on several occasions. Also, the temperature profile of the Linton Stream (mean = 17.8 °C; S.D. = 3.28; Figure 29) from the Magaguadavic River, displayed neither the extreme highs nor lows of the other sampling sites. Water pH levels at six sites were similar (pH 6-7) with the exception of Linton Stream which was more acidic (pH 5.5-6.5).

## **Discussion**

The analysis of unknown origin juvenile salmon from the Magaguadavic River, over a three-year period, indicated that a large proportion were of farmed origin. This finding is consistent with the results of Stokesbury and Lacroix (1997). In that study scale characteristics and external morphology were used to distinguish between farmed and native origin groups. They reported that the majority of smolts migrating from the Magaguadavic River in 1996 were of farmed origin.

In the present study the jackknife cross-validation indicated that 90.3% of the known origin juveniles were correctly classified to origin. This was done using scale characteristics established in the first six months of life. These scale characteristics span the time from initial scale formation (area of the focus) to the establishment of the 6<sup>th</sup> circuli. Therefore, this method was accurate in identifying the farmed or native origin of juvenile salmon by scale characteristics established in their age 0+ year. This may be previous to the establishment of definitive morphological characteristics of cultured origin.

When the discriminant function was applied to the “unknown origin” juveniles, the results also indirectly validated the method. First, of the predicted-farmed origin juveniles from the Magaguadavic River (1996-1998), 81% were located within a 5-km radius of one of the three commercial salmon aquaculture hatcheries.

Second, only a small proportion (8.8%) of the juveniles sampled in the Digdequash River, where the sampling site was approximately 30 km upstream from the hatchery, were of farmed origin. Only a small proportion of juveniles of farmed origin would be expected at this site because juvenile salmon do not often migrate large distances upstream (Clifford et al. 1998a; McCormick et al. 1998). Also, it should be noted that 8.8% is within the expected error of this method. Therefore, it is possible that none of these fish are of farmed origin.

Third, the percentage of predicted farmed and native origin juveniles sampled in the Waweig River where the collection site was located directly below the river's commercial hatchery operation, was considerably different from that of the Digdequash River. In the Waweig River, the discriminant function analysis predicted that a substantial proportion of the juveniles captured at this site were of farmed origin (41%). This indicates the importance of distance from a hatchery. The results in the Waweig River were consistent with those of the Magaguadavic River and provide support for the potential predictive power of the identification method.

#### *Scale Characteristics as Predictors of Origin*

Eight variables based on scale characteristics were significant predictors of the farmed or native origin of juvenile Atlantic salmon captured in the wild. The strongest predictor was the mean distance between the first six circuli. As predicted, farmed origin fish had a larger mean distance (14.25  $\mu\text{m}$ ) between the first six circuli than the native origin fish (11.29  $\mu\text{m}$ ). This is consistent with the results of Marcogliese and Casselman (1998) who found that the mean distance between circuli was greater for hatchery-reared than native rainbow trout.

The strength as predictors of the first five circuli pairs demonstrates how large the growth difference is between farmed and native salmon, especially at an early age. As predicted, the difference in spacing of each of the first five-circuli pairs was significantly larger for the farmed origin juveniles than for the native origin juveniles (Table 5). However, difference in the spacing of the sixth circuli pair was not significant. This may indicate that feeding rates and therefore growth rates in the wild equaled those in captivity at the time that the sixth circuli pair was established. This may have occurred in late July or August when food in the wild is plentiful. Possibly confounding the difference between the growth rate of farmed and native origin fish at this time.

The “Area of the Focus” was also a strong predictor of farmed or native origin. This variable has not previously been examined for its use as a predictor of origin in Atlantic salmon. The known farmed origin sample showed a significantly larger focal area ( $5752 \text{ um}^2$ ) than the native origin group ( $4411 \text{ um}^2$ ). This characteristic forms early (fork length = 25mm to 28mm) and is present for the entire life cycle of the fish. Therefore, it may be of great value in classifying Atlantic salmon to origin.

The standard deviation of the first six circuli was greater for the known origin native sample (3.30  $\text{um}$ ) than for the farmed origin sample (2.21  $\text{um}$ ). This was the first time that standard deviation of circuli spacing has been used to classify farmed and native origin juvenile salmonids. Standard deviation of circuli has been investigated (Marcogliese and Casselman 1998) but only as a measure of variance to quantify the mean. However, mean and variance in this situation measure very

different things. The standard deviation's strength as a predictor of origin shows that the growth rate of the native juvenile salmon in the wild varies much more than the growth rate of the farmed juveniles in culture.

### *Implications of the Study*

Juvenile escapees enter river systems from hatcheries at age 0+. Previous procedures may not have identified some or all of these escapees. For example, the two methods used by Stokesbury and Lacroix (1997) were accurate in identification of juvenile escapees after their first year. However, there were some inconsistencies in the results. The external examination method, based on size at a given age and appearance of fins, predicted that 67.2% of the smolts migrating from the river were juvenile escapees, and that only 23.4% were native origin smolts. The discriminant function method, based on the back calculated length at age one and the number of circuli in the first annual zone, predicted that 51.0% of the smolts were juvenile escapee origin while 39.6% were native origin. However, the discriminant function analysis probably overestimated the number of native smolts as it predicted that 30.8% of the smolts leaving the Magaguadavic River were 1-year-old native smolts (Stokesbury and Lacroix 1997). Previous studies indicated that the average smolt age of native adults returning to the Magaguadavic River was 80% age 2 and 20% age 3 (Martin, 1984). The Magaguadavic River system is not known to produce native 1-year-old smolts. Therefore, it follows that the method based on external signs of culture was more accurate in the identification of farmed juveniles than the discriminant function method.

External signs of cultured origin are more obvious in adult-farmed salmon than in juvenile farmed salmon (Fleming et al. 1994). Fin erosion is greater and growth advantage is larger with increased time spent in culture. Also, morphological convergence of farmed juveniles grown to adulthood in marine rearing and native adults may occur. Fleming et al. (1994) suggested this as environmentally induced characteristics of culture (i.e. head and trunk morphology) in body form of cultured juveniles were not found in adults. Therefore, although identification techniques based on morphological deformations caused by culture are successful when used to identify adult escapees, they may not be as successful at identifying juvenile escapees (Lund et al. 1989, cited in Lund and Hansen, 1991). Given this and the results of Stokesbury and Lacroix (1997), it may be that external examination of characteristic signs of culture more accurately identify cultured origin salmon than discriminant function analysis based on scale characteristics. However, this may be the case only if the salmon have spent enough time in culture to gain significant morphological characteristics of culture and, therefore, may not apply to age 0+ escapees. As most juvenile escapees entering rivers are age 0+, past studies may have underestimated the prevalence of escapees in the native juvenile population of rivers such as the Magaguadavic River. This may have implications for the management of native salmon stocks in rivers that support hatcheries.

Lacroix et al. (1998) identified the number of escapees entering the wild as a key factor in the genetic impact of escapees on native stocks. They state that spawning of past juvenile escapees would “greatly” accelerate the loss of the native stock through genetic introgression. Also, they suggest that the constant intrusion of

escapees (as occurred in the Magaguadavic River from 1996-1998) gives a false perception of robustness to a native population which may actually be dwindling. This may have important consequences for the management of these rivers.

Although it was beyond the scope of this study to provide a quantitative estimate of escapees from the hatcheries on the Magaguadavic River, results suggest that significant numbers of juvenile escapees were present in the native juvenile population of the Magaguadavic River in 1996 (34%), 1997 (63%), and 1998 (42%). These numbers are especially problematic given that juvenile escapees occurred repeatedly each year. That is, their presence was not the result of an isolated incident.

The three commercial aquaculture hatcheries on the Magaguadavic River produce in excess of 2 million smolts annually for the aquaculture industry (Stokesbury and Lacroix 1997). In contrast, a population model based on egg deposition estimates predicted that in 1996 about 7,100 native smolts were produced in the Magaguadavic River (Lacroix and Stokesbury (submitted)). Given that farmed juveniles escape into the wild system, this imbalance which is heavily in favor of the farmed population, may genetically and ecologically threaten the native stock. It would only take a small percentage of the farmed juveniles to escape into the wild to have a significant genetic effect on the native population (Hindar et al. 1991; Lacroix et al. 1998) which may be irreversible (Lacroix et al. 1998).

Taking into account recently reported fresh water survival rates for Atlantic salmon juveniles in Catamaran Brook, Miramichi River, New Brunswick (0+ to 1+ = 33%; 1+ to 2+ = 33%) (Cunjak and Therrien 1998) if farmed salmon escape at age 0+ and migrate as two year-old-smolts, only 3.2 % of the production of the hatcheries

would have to escape to match the estimated total native smolt production of the Magaguadavic River. This estimate is probably conservative as 32.25% of juvenile escapees in the Magaguadavic River smolt as one-year-olds (Stokesbury and Lacroix, 1997). Therefore, the river survival of juvenile escapees in the Magaguadavic River may be greater than that of the native fish in Catamaran Brook which have a longer fresh water residency.

When predicting the effect of hatchery escapees on native stocks it may be helpful to compare their intrusion into the wild ecosystem with other large-scale introductions of non-indigenous salmonids. Since the 1970's, the Department of Fisheries and Oceans Atlantic salmon hatchery programs have used wild broodstock for restoration of native populations (Goff 1996). However in The United States of America, the Pacific Northwest salmonid hatchery programs have often collected domesticated broodstock selected for hatchery condition and distributed them randomly in rivers (Goff 1996). This has resulted in the permanent loss of many native stocks (Holmes 1995). Therefore, the introduction of farmed escapees into the wild system without forethought of the founder effect, domestication and number or distribution may result in the permanent loss of native stocks.

#### *Limitations of Study*

There were a number of limitations of this study. Several assumptions were made because the discriminant function analysis was based on scale characteristics, which in turn reflect fish growth. In dealing specifically with the Magaguadavic River juvenile population, predicted farmed origin juveniles appear to congregate down river of hatcheries. Temperatures in some of these areas may be different from



other areas of the river further from hatcheries. Mean temperature at sites directly below hatchery installations were in one case lower (Waweig River, mean = 15.2 °C; S.D. = 5.91) and in one case higher (Linton Stream, mean = 17.8 °C; S.D. = 3.28) than the mean temperatures at sites not located downstream of hatcheries (Kedron Stream, mean = 16.3 °C; S.D. = 5.86; Digdequash River, mean = 16.2 °C; S.D. = 6.04) presumably because of the presence of hatchery effluent. As water temperature affects fish growth (Brett et al., 1969; Lagler et al. 1977; Riddle and Leggett 1981; Conover 1990) and hence scale characteristics, the presence of hatchery effluent may confound classification to natal origin of juveniles in areas directly below hatcheries.

The procedure used in this study was developed using juvenile salmon from the Magaguadavic River and Dennis Stream. However, it was used to classify unknown origin juvenile salmon from the Magaguadavic River and other nearby rivers (Digdequash River and Waweig River). This may have presented a problem because characteristics of salmon in different rivers vary (Riddell et al. 1981). However, because smolt age is directly linked to growth (Symons 1978), it can be used as an indicator of growth conditions in a river. Smolt ages from rivers in this region are similar. So, it was assumed possible to examine salmon from adjacent river systems using criteria established on juvenile salmon from the Magaguadavic River and Dennis Stream with minimal error in identification.

The presence of stocked fish also may have had a confounding effect on this study. Stocked fish are often the progeny of wild broodstock and have not undergone the artificial selection that multi-generational cultured fish have. Because their growth patterns tend to be intermediate to the growth patterns of farmed and native

origin fish, their origin may not have been accurately predicted by the discriminant function analysis. For example, in 1997 and 1998, predicted juvenile escapees were collected in the Kedron Stream (Figure 20). The Kedron Stream site was approximately 30 km from any hatchery and approximately 5 km up a side stream from the main stem of the river. It was an unlikely place to find cultured hatchery escapees because in the Magaguadavic River, 81% of the predicted juvenile escapees were captured within 5 km of a hatchery. The Kedron Stream and connecting Piscahagen Stream were stocked in 1997 with “ranched” fingerlings, the progeny of wild Magaguadavic River parents (Carr and Whoriskey 1998). In the past, stocked salmon may have entered the Kedron Stream from the nearby Piscahagen Stream (Smith and McGonigle 1935). Stocked fish captured in the Kedron Stream were identified by their adipose fin clips and removed from the data set. It is possible that through improper techniques of marking not all the stocked fish were marked. Therefore, some of the predicted “farmed origin” juveniles from the Kedron Stream in 1997 and 1998 may actually have been stocked fish placed in the Kedron and Piscahagen Streams in 1997.

Finally, in order to extrapolate the findings of this study to the entire juvenile salmon populations of the rivers, sampling would have had to have been random so that every individual in the population would have an equal chance of being selected (Brown and Austen 1996). The site selection, and therefore the sampling in this study, was not random. As a result, the predicted percentages may not be taken as representative of the entire juvenile Atlantic salmon populations of the rivers.

### *Conclusions*

Through the use of discriminate function analysis based solely on scale characteristics, the origin of age 0+ juvenile Atlantic salmon can be predicted 90.3% of the time. This was accomplished using a procedure that was both economical and had little impact on the wild salmon population.

The growth patterns of farmed and native salmon, which were recorded on their scales, differed significantly. The different developmental forces experienced in the first year of life by these two groups, in effect, left characteristics that would allow classification of their farmed or native origin at any age. The predictors of origin used in this procedure would be present on all of the fish produced at this time, in this system.

Unlike phenotypic examination procedures, this study provided a quantifiable measure of accuracy through its jackknife cross-validation. Procedures of classification of fish origin that depend solely on phenotypic characteristics have no assessment of accuracy. For example, it may be true that all fish with extensive fin erosion or mutations are of farmed origin. However, this does not mean that all farmed origin fish show extensive fin erosion or mutations. Without a measure of accuracy, such as that provided in this study by the jackknife cross-validation, assessment of origin can become subjective.

Through the use of this procedure, groups of rivers that produce smolts of the same age, with similar life histories, may be evaluated. Through the development of similar models on strategic river systems, overall assessment of the farmed or native origin of juvenile salmon produced in rivers may be determined.

## References

- Allendorf, F. W. and Phelps, S. R. 1981. Use of allelic frequencies to describe population structure. *Can. J. Fish. Aquat. Sci.* 38: 1507-1514.
- Amiro, P. G. 1998. The mystery of the missing inner Bay of Fundy salmon. *Atl. Sal. J.*, Autumn 1998, pp. 50-53.
- Alverson, D. L. and Ruggerone, G. T. 1997. Escaped farm salmon: environmental and ecological concerns. In *Salmon aquaculture review*, vol. 3: Technical Advisory Team Discussion Paper B. British Columbia Environmental Assessment Office, Victoria, B.C.
- Bagliniere, J. L. and Maise, G. 1985. Precocious maturation and smoltification in wild Atlantic salmon in the Armorican Massif, France. *Aquaculture*, 45: 249-263.
- Bailey, J. 1998. Options for containment of farmed Atlantic salmon. Dept. Fish. And Oceans Can. Stock. Assess. Secretariat Res. Doc. 98/165
- Bams, R. A. 1976. Survival and propensity for homing as affected by presence or absence of locally adapted paternal genes in two transplanted populations of pink salmon (*Oncorhynchus gorbuscha*). *J. Fish. Res. Bd. Can.* 33: 456-485.
- Baum, E. 1997. *Maine Atlantic Salmon: a national treasure*. Atlantic Salmon Unlimited, Herman, Maine, U.S.A.
- Baum, E. T. 1998. History and description of the aquaculture industry in Maine. Dept. Fish. And Oceans Can. Stock. Assess. Secretariat Res. Doc. 98/152

- Beacham, T. D. and Evelyn, T. P. T. 1992. Genetic variation in disease resistance and growth of Chinook, Coho, and Chum salmon with respect to Vibriosis, Furunculosis, and bacterial kidney disease. *Trans. Am. Fish. Soc.* 121: 456-485.
- Berejikian, B. A. 1995. The effects of hatchery and wild ancestry and experience on the relative ability of steelhead trout fry (*Oncorhynchus mykiss*) to avoid a benthic predator. *Can. J. Fish. Aquat. Sci.* 52: 2476-2482.
- Berejikian, B. A., Mathews, S. B., and Quinn, T. P. 1996. The effects of hatchery and wild ancestry on the development of agnostic behaviour in steelhead trout fry (*Oncorhynchus mykiss*). *Can. J. Fish. Aquat. Sci.* 53: 2004-2014.
- Bernard, R. L., and Myers, K. W. 1996. The performance of quantitative scale pattern analysis in the identification of hatchery and wild steelhead (*Oncorhynchus mykiss*). *Can. J. Fish. Aquat. Sci.* 53: 1727-1735.
- Brett, J. R., Shelbourn, J. E., and Shoop, C. T. 1969. Growth rate and body composition of fingerling sockeye salmon, *Oncorhynchus nerka*, in relation to temperature and ration size. *J. Fish. Res. Bd. Can.* 26: 2363-2394.
- Brown, M. L., and Austen, D. J. 1996. Data management and statistical techniques. Pages 17 – 62 *In* B. R. Murphy and D. W. Willis, editors. *Fisheries techniques*, 2nd edition. American Fisheries Society, Bethesda, Maryland. 731p.
- Caillet, G. M., Love, M. S., and Ebeling, A. W. 1986. *Fishes: A field and laboratory manual to their structure, identification and natural history.* Waveland Press Inc. Illinois. 194 p.

- Carr, J. W. 1995. Interactions between wild and aquaculture Atlantic salmon in the Magaguadavic River, New Brunswick. M. Sc. Thesis, University of New Brunswick, Fredericton, 77 pp.
- Carr, J. W., Anderson, J. M., Whoriskey, F. G. and Dilworth, T. 1997a. The occurrence and spawning of cultured Atlantic salmon (*Salmo salar*) in a Canadian river. ICES J. Mar. Sci. 54: 1064-1073.
- Carr, J. W., G. L. Lacroix, J. M. Anderson, and T. Dilworth. 1997b. Movements of non-maturing cultured Atlantic salmon (*Salmo salar*) in a Canadian river. ICES J. Mar. Sci. 54: 1082-1085.
- Carr, J. W. and Whoriskey, F. G. 1998. Atlantic salmon (*Salmo salar*) in the Magaguadavic River, New Brunswick, 1992-1997. Report of the Atlantic salmon Federation, St. Andrews, New Brunswick.
- Chang, B. D. 1998. The salmon aquaculture industry in the Maritime provinces. Can. Stock. Assess. Secretariat Res. Doc. 98/151
- Conover, D. O. 1990. The relation between capacity for growth and length of growing season: Evidence for and implications of countergradient variation. Trans. Am. Fish. Soc. 119: 416-430.
- Cunjak, R. A., Chadwick, E. M. P., and Shears, M. 1989. Downstream movements and estuarine residence by Atlantic salmon parr (*Salmo salar*). Can. J. Fish. Aquatic Sci. 46: 1466-1471.
- Cunjak, R. A., and Therrien, J. 1998. Inter-stage survival of wild juvenile Atlantic salmon, *Salmo salar* L. Fish. Manag. Ecol. 5: 209 – 223.

- Clifford, S. L., McGinnity, P. and Ferguson, A. 1998a. Genetic changes in an Atlantic salmon population resulting from escaped juvenile farm salmon. *J. Fish Biol.* 52: 118-127.
- Clifford, S. L., McGinnity, P. and Ferguson, A. 1998b. Genetic changes in Atlantic salmon (*Salmo salar*) populations of Northwest Irish rivers resulting from escapes of adult farm salmon. *Can. J. Fish. Aquat. Sci.* 55: 358 - 363
- Cross, T. F., and King, J. 1983. Genetic effects of hatchery rearing in Atlantic salmon. *Aquaculture* 33: 33-40.
- Crozier, W. W. 1991. Report on scientific examination of presumed fish farm escaped Atlantic salmon recovered from the Co. Antrim coast and the Glenarm River. Dept. Agric., N. Ireland, International report, 11p.
- Crozier, W. W. 1993. Evidence of genetic interaction between escaped farmed salmon and wild Atlantic salmon (*Salmo salar* L.) in a northern Irish river. *Aquaculture.* 113: 19-29.
- Crozier, W. W. 1998. Incidence of escaped farmed salmon, *Salmo salar* L., in commercial salmon catches and fresh water in Northern Ireland. *Fish. Manag. Ecol.* 5: 23-29.
- deVries, D. R. and Frie, R. V. 1996. Determination of age and growth. Pages 483-512 in B. R. Murphy and D. W. Willis, editors. *Fisheries techniques*, 2<sup>nd</sup> edition. American Fisheries Society, Bethesda, Maryland. 731 p.
- Einum, S. and Fleming, I. A. 1997. Genetic divergence and interactions in the wild among native, farmed and hybrid Atlantic salmon. *J. Fish Biol.* 50: 634-651.

- Ewing, R. D., and Ewing, S. K. 1995. Review of the effects of rearing density on survival to adulthood for Pacific salmon. *Prog. Fish-Cult* 57:1-25.
- Fevolden, S. E., Refstie, T. and Roed, K. H. Selection for high and low cortisol stress response in Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 95:53-65.
- Fevolden, S. E., Nordmo, R., Refstie, T. and Roed, K. H. 1993. Disease resistance in Atlantic salmon (*Salmo salar*) selected for high or low responses to stress. *Aquaculture*, 109:215-224.
- Fjalestad, K. T., Gjedrem, T., and Gjerde, B. 1993. Genetic improvement of disease resistance in fish: an overview. *Aquaculture* 111: 65-74.
- Fjalestad, K. T., Larsen, H. J. and Roed, K. H. 1996. Antibody response in Atlantic salmon (*Salmo salar*) against *Vibrio anguillarum* and *Vibrio salmonicida* O-antigens: Heritabilities, genetic correlations and correlations with survival. *Aquaculture* 145: 77-89.
- Fleming, I. A. and Gross, M. R. 1992. Reproductive behaviour of hatchery and wild coho salmon (*Oncorhynchus kisutch*): does it differ? *Aquaculture* 103: 101-121.
- Fleming, I. A., Jonsson, B., and Gross, M. R. 1994. Phenotypic divergence of sea-ranched, farmed and wild salmon. *Can. J. Fish. Aquat. Sci.* 51: 2808-2824.
- Fleming, I. A., Jonsson, B., Gross, M. R., and Lamberg, A. 1996. An experimental study of the reproductive behavior and success of farmed and wild Atlantic salmon (*Salmo salar* L.). *J. Appl. Ecol.* 33: 893-905.



- Fleming, I. A. and Einum, S. 1997. Experimental tests of genetic divergence of farmed from wild Atlantic salmon due to domestication. *ICES J. Mar. Sci.* 54: 1051-1063.
- Friars, G. W., Bailey, J. K., and Coombs, K. A. 1990. Correlated responses to selection for grilse length in Atlantic salmon. *Aquaculture* 85: 171-176.
- Friars, G. W., Bailey, J. K., and O'Flynn, F. M. 1995. Application of selection for multiple traits in cage reared Atlantic salmon (*Salmo salar*). *Aquaculture* 137: 213-217.
- Gausen, D. and Moen, V. 1991. Large scale escapes of Atlantic salmon (*Salmo salar*) into Norwegian rivers threaten natural populations. *Can. J. Fish. Aquat. Sci.* 48: 426-428.
- Gjedrem, T., Gudgeon, H. M. and Gjerde, B. 1991. Genetic origin of Norwegian farmed Atlantic salmon. *Aquaculture* 98: 41-50.
- Goff, T. 1996. An overview of hatcheries in the 90's: Past abuses, present successes, and future promise. Report for the diadromous division, Dept. Fish. And Oceans. Mactaquac, New Brunswick.
- Gross, M. R. 1998. One species with two biologies: Atlantic salmon (*Salmo salar*) in the wild and aquaculture. *Can. J. Fish. And Aquat Sci.* 55 (Suppl. 1): 131-144.
- Hansen, L. P., and Jonsson, B. 1991. Evidence of a genetic component in the seasonal return pattern of Atlantic salmon, *Salmo salar* L. *J. Fish Bio.* 38: 251-258.

- Hansen, L. P., Jacobsen, J. A. and Lund, R. A. 1993. High numbers of farmed Atlantic salmon, *Salmo salar* L., observed in oceanic waters north of the Faroe Islands. *Aqua. Fish. Manag.* 24: 777-781.
- Hansen, L. P., Windsor, M. L., and Youngson, A. F. 1997. Interactions between salmon culture and wild stocks of Atlantic salmon: The scientific and management issues. *ICES J. Mar. Sci.* 54: 963-964.
- Hastein, T., and Lindstad, T. 1991. Disease in wild and cultured salmon: possible interactions. *Aquaculture* 98: 277-288
- Hesthagen, T., Hegge, O., Skurdal, J. and Dervo, B. K. 1995. Differences in habitat utilization among native, native stocked, and non-native stocked brown trout (*Salmo trutta*) in a hydroelectric reservoir. *Can. J. Fish. Aquat. Sci.* 52: 2159-2167.
- Hiilivirta, P., Ikonen, E., and Lappalainen, J. 1998. Comparison of two methods for distinguishing wild from hatchery-reared salmon (*Salmo salar* Linnaeus, 1758) in the Baltic Sea. *ICES J. Mar. Sci.* 55: 981-986.
- Hindar, K., Ryman, N., and Utter, F. 1991. Genetic effects of cultured fish on natural fish populations. *Can. J. Fish. Aquat. Sci.* 48: 945-957.
- Holmes, B. 1995. Saving Snake River's wild salmon. *New Scientist*, April: 2p
- Hutchings, J. A. and Jones, M. E. B. 1998. Life history variation and growth rate thresholds for maturity in Atlantic salmon, *Salmo salar*. *Can. J. Fish. Aquat. Sci.* 55 (Suppl. 1): 22-47.
- Hvidsten, N. A. and Lund, R. A. 1988. Predation on hatchery reared and wild smolts of Atlantic salmon. *J. Fish Biol.* 33: 121-126.

- Ikonen, E., Hiilivirta, P. and Lappalainen, J. 1991. Report of the Baltic salmon scale reading workshop, Utsjoki, Finland, 15-17 January 1991. ICES Cooperative Research Report no. 192, 71pp.
- Johnson, B. O., and Jensen, A. J. 1991. The Gyrodactylus story in Norway, *Aquaculture*, 98: 289-302.
- 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 in Alberni Inlet, British Columbia. *Can. J. Fish. Aquat. Sci.* 53: 2888-2897.
- Jonasson, J. 1996. Selection experiments on Atlantic salmon ranching. II. Variation among release sites and strains for return rate, body weight and ratio of grilse to total return. *Aquaculture* 144: 277-294.
- Koljonen, M. L. and Pella, J. J. 1997. The advantage of using smolt age with allozymes for assessing wild stock contributions to Atlantic salmon catches in the Baltic Sea. *ICES J. Mar. Sci.* 54: 1015-1030.
- Korman, J., Bravender, B., and Levings, C. D. 1997. Utilization of the Campbell River estuary by juvenile Chinook salmon (*Oncorhynchus tshawytscha*) in 1994. *Can. Tech. Rep. Fish. Aquat. Sci.*, No. 2169. 45pp
- Lacroix, G. L., Galloway, B. J., Knox, D., and MacLatchy, D. 1997. Absence of seasonal changes in reproductive function of cultured Atlantic salmon migrating into a Canadian river. *ICES J. Mar. Sci.* 54: 1086-1091.
- Lacroix, G. L., Korman, J., and Heath, D. D. 1998. Genetic introgression of the domestic Atlantic salmon genome into wild populations: a simulation of

requirements for conservation. Can. Stock Assess. Secretariat Res. Doc.  
98/158

Lacroix, G. L., and Stokesbury, M. J. W. (submitted). Marine survival of Atlantic salmon escaping from commercial hatcheries relative to the wild population in a Canadian river. ICES J. Mar. Res.

Lagler, K. F., Bardach, J. E., Miller, R. R. and May Passino, D. R. 1977.

Ichthyology, 2ed. John Wiley and Sons, Toronto. 505 p.

Lear, W. H. and Misra, R. K. 1978. Clinal variation in scale characteristics of Atlantic salmon (*Salmo salar*) based on discriminate function analysis. J. Fish. Res. Bd. Can. 35: 43-47

Lund, R. A., Hansen, L. P. and Jarvi, T. 1989. Identifisering av oppdrettslaks og vill-laks ved ytre morfologi, finnestorrelse og skjellkarakterer (Identification of reared and wild salmon by external morphology, size of fins and scale characteristics). NINA Forskningsrapport 1, 1-54. (In Norwegian with English summary.)

Lund, R. A., and Hansen, L. P. 1991. Identification of wild and reared Atlantic salmon, *Salmo salar* L., using scale characteristics. Aquacul. Fish. Manag. 22: 499-508.

Lund, R. A., Okland, F., and Hansen, L. P. 1991. Farmed Atlantic salmon (*Salmo salar* L.) in fisheries and rivers in Norway. Aquaculture 98: 143-150.

Lura, H. and Saegrov, H. 1991. Documentation of successful spawning of escaped farmed female Atlantic salmon, *Salmo salar*, in Norwegian rivers. Aquaculture 98: 151-159.

- Mac Arthur, R. H. and Wilson, E. O. 1967. The theory of island biogeography. Monographs on population biology, Levin, S. A. and Horn, H. S. eds. Princeton University Press, New Jersey
- Marcogliese, L. A. and Casselman, J. M. 1998. Scale methods for discriminating between Great Lakes stocks of wild and hatchery trout, with a measure of natural recruitment in Lake Ontario. *N. Am. J. Fish. Manag.* 18: 253-268
- Marshall T. L. and Jones, R. 1996. Status of Atlantic salmon stocks in the Saint John River and Southwest New Brunswick, 1995. DFO Atl. Fish. Res. Doc. 96/40
- Martin, J. D. 1984. Atlantic salmon and alewife passage through a pool and weir fishery on the Magaguadavic River. New Brunswick, during 1983. *Can. Man. Rep. Fish Aquat. Sci.*, No. 1776
- Mayr, E. 1942. Systematics and the origin of species. Columbia University Press.
- McCormick, S. D., Hansen, L. P., Quinn, T. P. and Saunders, R. L. 1998. Movement, migration, and smolting of Atlantic salmon (*Salmo salar*). *Can. J. Fish. Aquat. Sci.* 55(Suppl. 1): 77-92.
- McGinnity, P., Stone, C., Taggart, J. B., Cooke, D., Cotter, D., Hynes, R., McCamley, C., Cross, T., and Ferguson, A. 1997. Genetic impact of escaped farmed Atlantic salmon (*Salmo salar* L.) on native populations: use of DNA profiling to assess freshwater performance of wild, farmed, and hybrid progeny in a natural river environment. *ICES J. Mar. Sci.* 54: 998 – 1008.
- McIssac, D. O., and Quinn, T. P. 1988. Evidence for a hereditary component in homing behaviour of chinook salmon (*Oncorhynchus tshawytscha*). *Can. J. Fish. Aquat. Sci.* 45: 2201-2205.

- Nicieza, A. G., Reiriz, L., and Brana, F. 1994. Variation in digestive performance between geographically disjunct populations of Atlantic salmon: countergradient in passage time and digestion rate. *Oecologia* 99: 243-251.
- Orciari, R. D., and Leonard, G. H. 1996. Length characteristics of smolts and timing of downstream migration among three strains of Atlantic salmon in a southern New England stream. *N. Am. J. Fish. Manage.* 16: 588-606.
- Petersson, E. and Jarvi, T. 1997. Reproductive behaviour of sea trout (*Salmo trutta*) – the consequences of sea ranching. *Behaviour*, 134: 1-22.
- Power, G. 1964. A technique for preparing scale smears. *Trans. Am. Fish. Soc.* 93: 201-202.
- Power, G. 1969. *The salmon of Ungava Bay*. Arctic Inst. N. Am. Tech. Pap. 22: 72p.
- Riddell, B. E. and Leggett, W. C. 1981. Evidence of an adaptive basis for geographic variation in both morphology and time of downstream migration of juvenile Atlantic salmon (*Salmo salar*). *Can. J. Fish. Aquat. Sci.* 38: 308-320
- Riddell, B. E., Leggett, W. C. and Saunders, R. I. 1981. Evidence of adaptive polygenic variation between two populations of Atlantic salmon (*Salmo salar*) native to tributaries of the S. W. Miramichi River, N. B. *Can. J. Fish. Aquat. Sci.* 38: 321 –333.
- Saunders, R. L. 1991. Potential interaction between cultured and wild Atlantic salmon. *Aquaculture* 98: 51-60.

- Schwartzberg, M. and Fryer, J. K. 1993. Identification of hatchery and naturally spawning stocks of the Columbia basin spring Chinook salmon by scale pattern analysis. *N. Am. J. Fish. Man.* 13: 263-271.
- Skaala, O., Dahle, G., Jorstad, K. E. and Naevdal, G. 1990. Interactions between natural and farmed fish populations: information from genetic markers. *J. Fish Biol.* 36: 449-460.
- Skaala, O., Jorstad, K. E. and Borgstrom, R. 1996. Genetic impact on two wild brown trout (*Salmo trutta*) populations after release of non-indigenous hatchery spawners. *Can. J. Fish. Aquat. Sci.* 55: 2027-2035.
- Smith, M. W. and M'Gonigle, R. H. 1935. The salmon potentialities of the Magaguadavic River System. Report to the Director, Atlantic Biological Station, St. Andrews, N. B.
- Soto, C. G. and Burhanuddin, B. 1995. Clove oil as a fish anesthetic for measuring length and weight of rabbitfish (*Siganus lineatus*). *Aquaculture* 136: 149-152.
- Stokesbury, M. J. and Lacroix, G. L. 1997. High incidence of hatchery origin Atlantic salmon in the smolt output of a Canadian river. *ICES J. Mar. Sci.* 54: 1074-1081.
- Symons, P. E. K. 1978. Estimated escapement of Atlantic salmon (*Salmo salar*) for maximum smolt production in rivers of different productivity. *J. Fish. Res. Bd. Can.* 36: 132 – 140.
- Symons P. E. K. and Martin, J. D. 1978. Discovery of juvenile Pacific salmon (Coho) in a small coastal stream of New Brunswick. *Fish. Bull.* 76: 487-489.

- Tabachnick, B. G. and Fidell, L. S. 1989. **Using multivariate statistics 2<sup>nd</sup> ed.**  
Harper Collins Publishers, Inc. New York, 746 p.
- Thorpe, J. E. 1991. **Acceleration and deceleration effects of hatchery rearing on salmonid development, and their consequences for wild stocks.** *Aquaculture* 98: 111-118.
- Unwin, M. J., and Lucas, D. H. 1993. **Scale characteristics of wild and hatchery chinook salmon (*Oncorhynchus tshawytscha*) in the Rakaia River, New Zealand, and their use in stock identification.** *Can. J. Fish. Aquat. Sci.* 50: 2475-2483.
- Verspoor, E. and Hammar, J. 1991. **Introgressive hybridization in fishes: the biochemical evidence.** *J. Fish Biol.* 39(Supp. A): 309-334.
- Webb, J. H., Hay, D. W., Cunningham, P. D., and Youngson, A. F. 1991. **The spawning behavior of escaped farmed and wild Atlantic salmon (*Salmo salar* L.) in a northern Scottish river.** *Aquaculture* 98: 97-110.
- Whoriskey, F. G., Lacroix, G. L., Carr, J., and Stokesbury, M. J. 1998. **A review and update of aquaculture impact studies carried out on the Magaguadavic River, southern Bay of Fundy, New Brunswick.** Dept. Fish. and Oceans Can. Stock Assess. Secretariat Res. Doc. 98/155.
- Whoriskey, F. G. 1999. **A bitter, bitter blow.** *Atl. Sal. J.* 48(4): 12-14
- Youngson, A. F., Buck, R. J. G., Simpson, T. H., and Hay, D. W. 1983. **The autumn and spring emigrations of juvenile Atlantic salmon, *Salmo salar* L., from the Girnock Burn, Aberdeenshire, Scotland: environment release of migration.** *J. Fish. Biol.* 23: 625-639.



- Youngson, A. F., Martin, S. A. M., Jordan, W. C., and Verspoor, E. 1991. Genetic protein variation in Atlantic salmon in Scotland: comparison of wild and farmed fish. *Aquaculture* 98: 231-242.
- Zhang, Z., Beamish, R. J., and Ridell, B. E. 1995. Differences in otolith microstructure between hatchery-reared and wild chinook salmon (*Oncorhynchus tshawytscha*). *Can. J. Fish. Aquat. Sci.* 52: 344-352.

**Table 1. Studies using scale characteristics (or data calculated from scale characteristics) as predictor variables in discriminant function analysis to classify Atlantic salmon to groups of natal origin or location.**

Study; Objective; Species; Developmental stage	Independent variables (scale characteristics or derived from scale characteristics)
Lear and Misra 1978 Clinal variation <i>Salmo salar</i> Adults	<ol style="list-style-type: none"> <li>1. Number of circuli in first annual river zone</li> <li>2. Number of circuli in second annual river zone</li> <li>3. Smolt age</li> <li>4. Number of circuli in first annual marine zone</li> </ol>
Ikonen et al. 1991 Cultured or native origin <i>Salmo salar</i> Adults	<ol style="list-style-type: none"> <li>1. Width of the first freshwater zone.</li> <li>2. Number of circuli in the first annual zone.</li> <li>3. Mean number of circuli per annual zone in freshwater phase.</li> </ol>
Stokesbury and Lacroix 1997 Cultured or native origin <i>Salmo salar</i> Juveniles	<ol style="list-style-type: none"> <li>1. Number of circuli in first annual zone.</li> <li>2. Back-calculated fork length at age one.</li> </ol>
Hiilivirta et al. 1998 Cultured or native origin <i>Salmo salar</i> Adults	<ol style="list-style-type: none"> <li>1. Width of the first freshwater annual zone.</li> <li>2. Maximum number of circuli per year in the freshwater zone.</li> <li>3. Mean distance between circuli in the freshwater zone.</li> </ol>

Table 2. Source of Atlantic salmon juveniles from which scales were used for the discriminant function analysis.

Origin	Sample Size	Year Collected	River
Known Hatchery	32	1997	Magaguadavic River, Connors Bros. Hatchery
Known Hatchery	35	1997	Magaguadavic River, Stolt Sea Farms Hatchery
Clipped (Ranched)	19	1998	Magaguadavic River, Kedron Stream (Stocked)
Known Wild	10	1996	Magaguadavic River, Lower Trout Brook
Known Wild	11	1996	Magaguadavic River, Kedron Stream
Known Wild	9	1996	Magaguadavic River, Piscahagen Stream
Known Wild	27	1998	Dennis Stream
Unknown	123	1996	Magaguadavic River
Unknown	143	1997	Magaguadavic River
Unknown	79	1998	Magaguadavic River, Kedron Stream
Unknown	37	1998	Magaguadavic River, Linton Stream
Unknown	11	1998	Magaguadavic River, Tomaston Corner
Unknown	87	1998	Digdequash River
Unknown	97	1998	Waweig River

Table 3. Results of a logistic regression performed on variables measured from scale characteristics of Atlantic salmon juveniles from the Magaguadavic River (N=30) and Dennis Stream (N=27), that were used to form the “Known wild” group used in the discriminant function analysis, B = the slope of the regression line. Measurements are for both river groups combined.

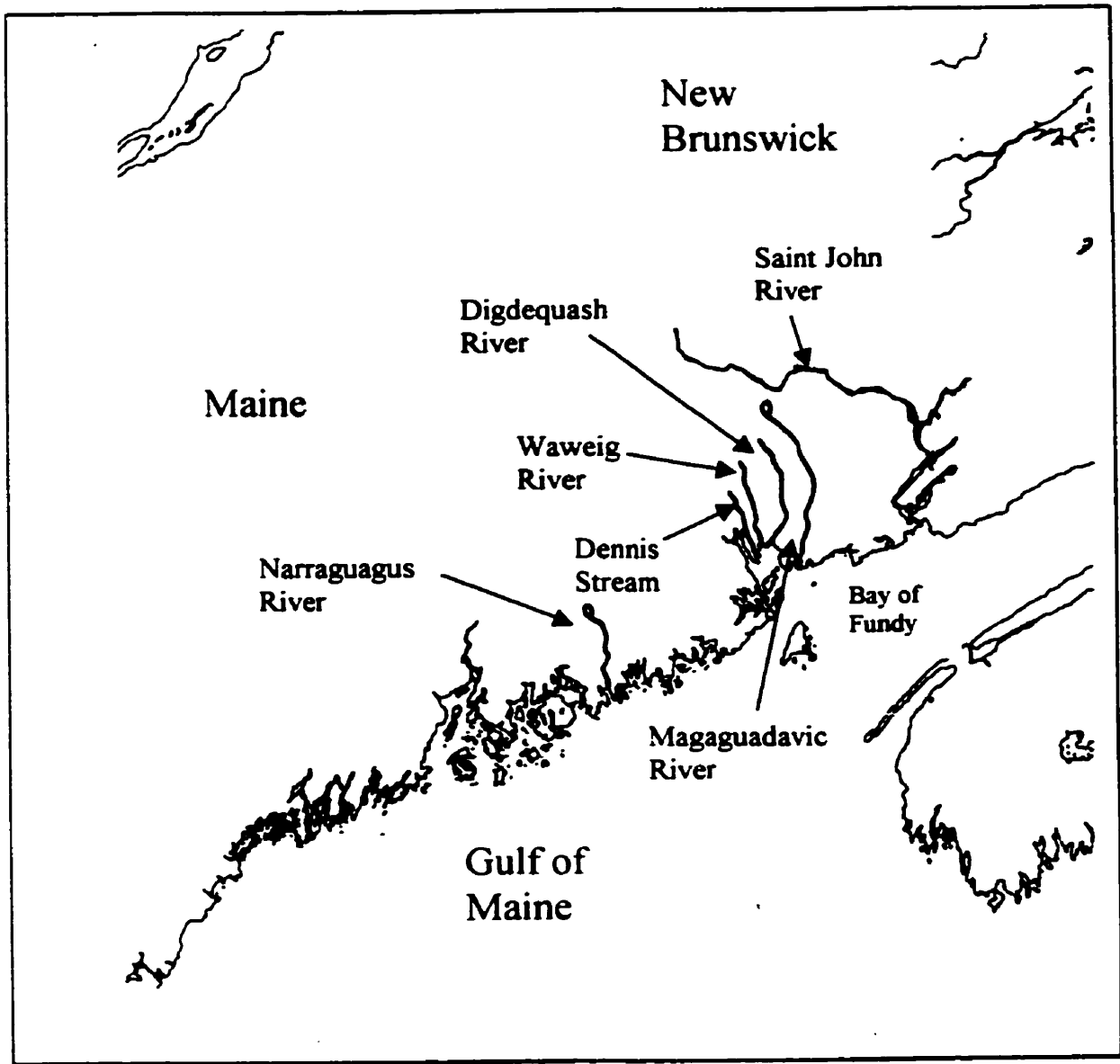
Measured Predictors (units)	Mean (SD)	B	S.E.	Sig.
Distance between Circuli pair 1 (um)	12.92 (3.99)	-0.17	0.11	0.13
Distance between Circuli pair 2 (um)	11.05 (3.35)	-0.01	0.09	0.93
Distance between Circuli pair 3 (um)	10.40 (2.83)	-0.04	0.12	0.71
Distance between Circuli pair 4 (um)	10.60 (3.62)	-0.02	0.10	0.86
Distance between Circuli pair 5 (um)	10.78 (3.60)	-0.00	0.14	0.99
Distance between Circuli pair 6 (um)	11.59 (3.24)	0.05	0.07	0.51
Area of the Focus (um <sup>2</sup> )	4411 (978)	-0.00	0.00	0.72
Mean of the Distance between the first 6 circuli (um)	11.29 (2.21)	0.28	0.42	0.51
Standard deviation of the distance between the first 6 circuli (um)	3.30 (1.20)	0.58	0.26	0.03

Table 4. Significance of predictors of group origin (hatchery or wild) of Atlantic salmon juveniles from the Magaguadavic River as computed by a discriminant function analysis using hatchery parr as a “known hatchery” group (N = 67) and wild parr from the Magaguadavic river (1996) and Dennis Stream (1998) as a “known wild” group (N = 57).

\*Not a significant predictor of group origin at  $p < 0.05$ .

Measured Predictors	Wilks' Lambda	F	Sig.
Distance between Circuli pair 1	.85	.28	<.001
Distance between Circuli pair 2	.63	.51	<.001
Distance between Circuli pair 3	.70	.44	<.001
Distance between Circuli pair 4	.78	.36	<.001
Distance between Circuli pair 5	.81	.32	<.001
Distance between Circuli pair 6	.98		.158*
Area of the Focus	.68	.46	<.001
Mean of the Distance between the first 6 circuli	.60	.55	<.001
Standard deviation of the distance between the first 6 circuli	.80	-.34	<.001





**Figure 1. Rivers of southwestern New Brunswick and Maine from which juvenile Atlantic salmon were sampled and smolt age statistics were used.**

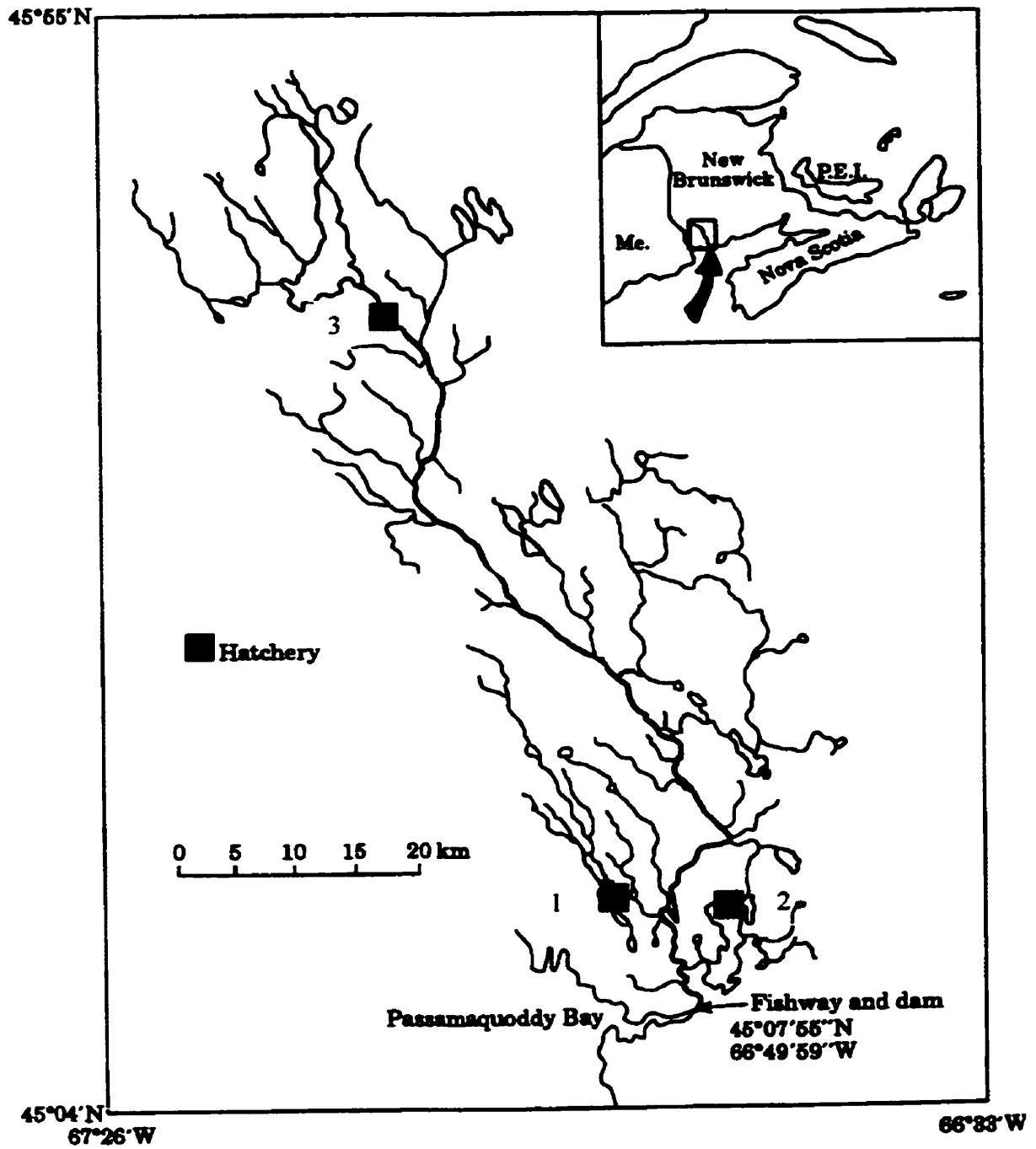


Figure 2. Commercial Atlantic salmon aquaculture hatcheries on the Magaguadavic River system, southwestern New Brunswick; 1 = Stolt Sea Farms hatchery; 2 = Connors Brothers Ltd. hatchery; 3 = Tomlinson hatchery



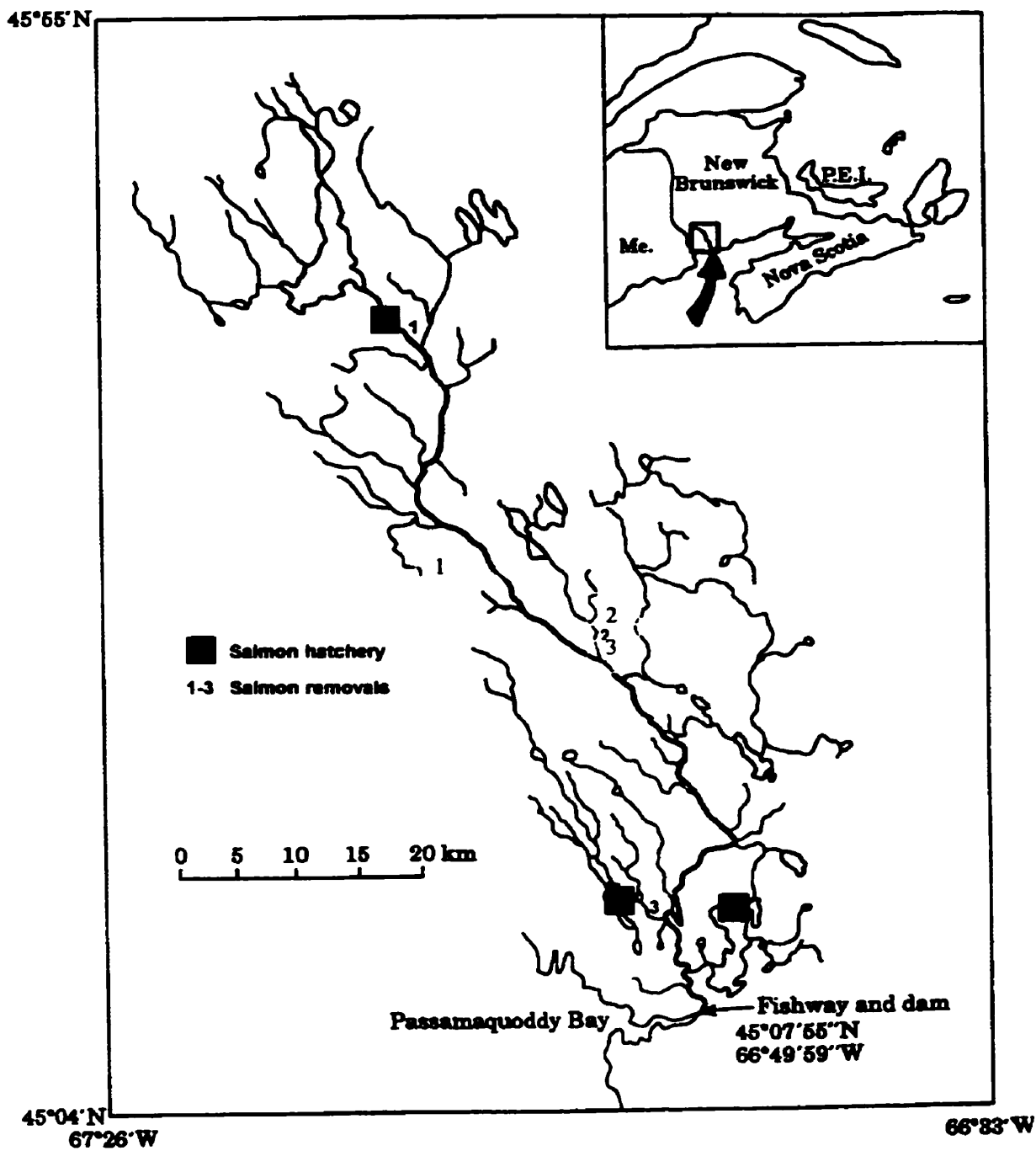


Figure 3. Native origin juvenile Atlantic salmon removals from the Magaguadavic River, New Brunswick in 1998 to be used in the “known origin native” group to help compute the discriminate function (1 = lower Trout Brook; 2 = Kedron Stream; 3 = Piscahagen Stream).

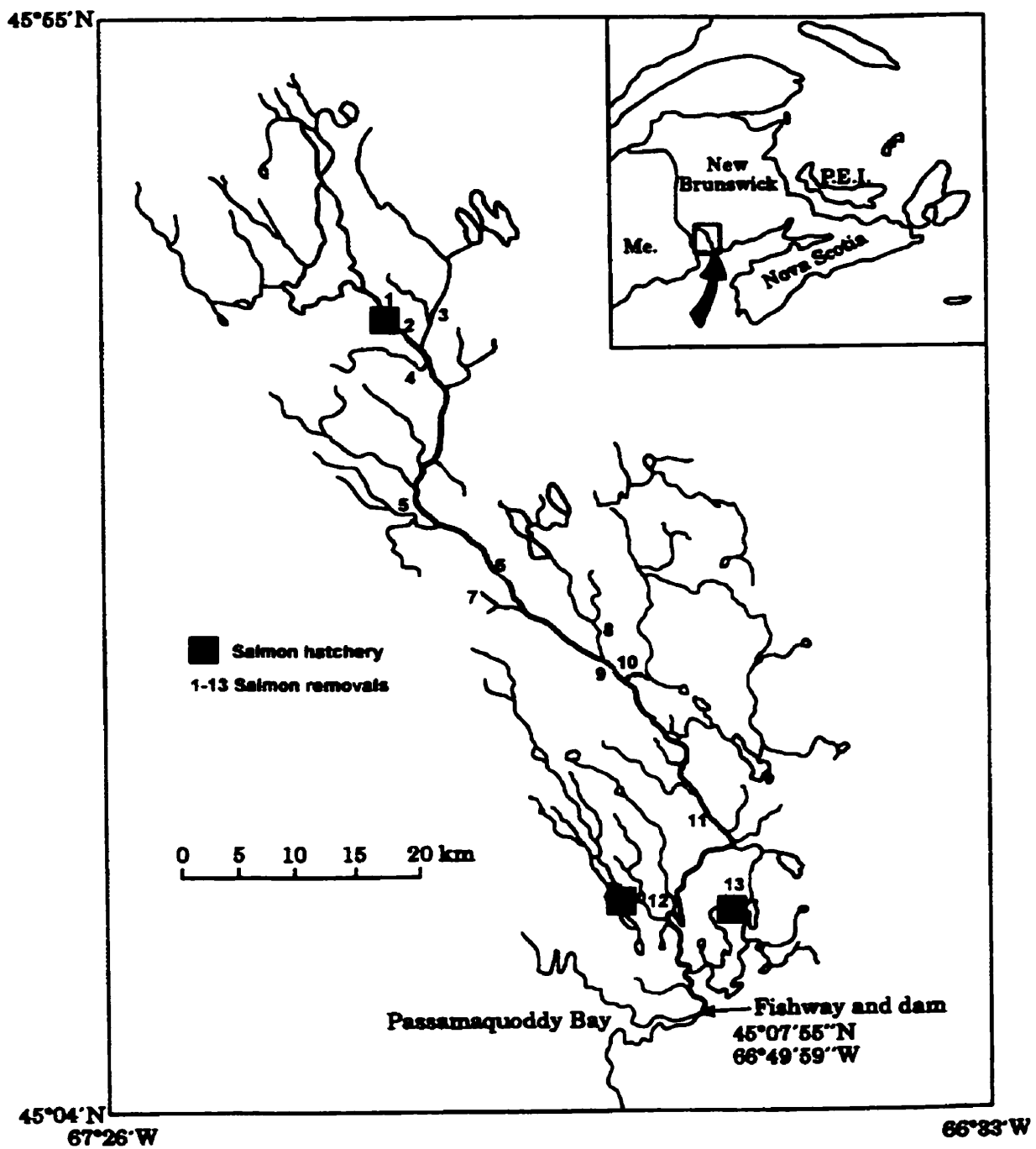


Figure 4. Wild juvenile Atlantic salmon removals from the Magaguadavic River, New Brunswick in 1996.

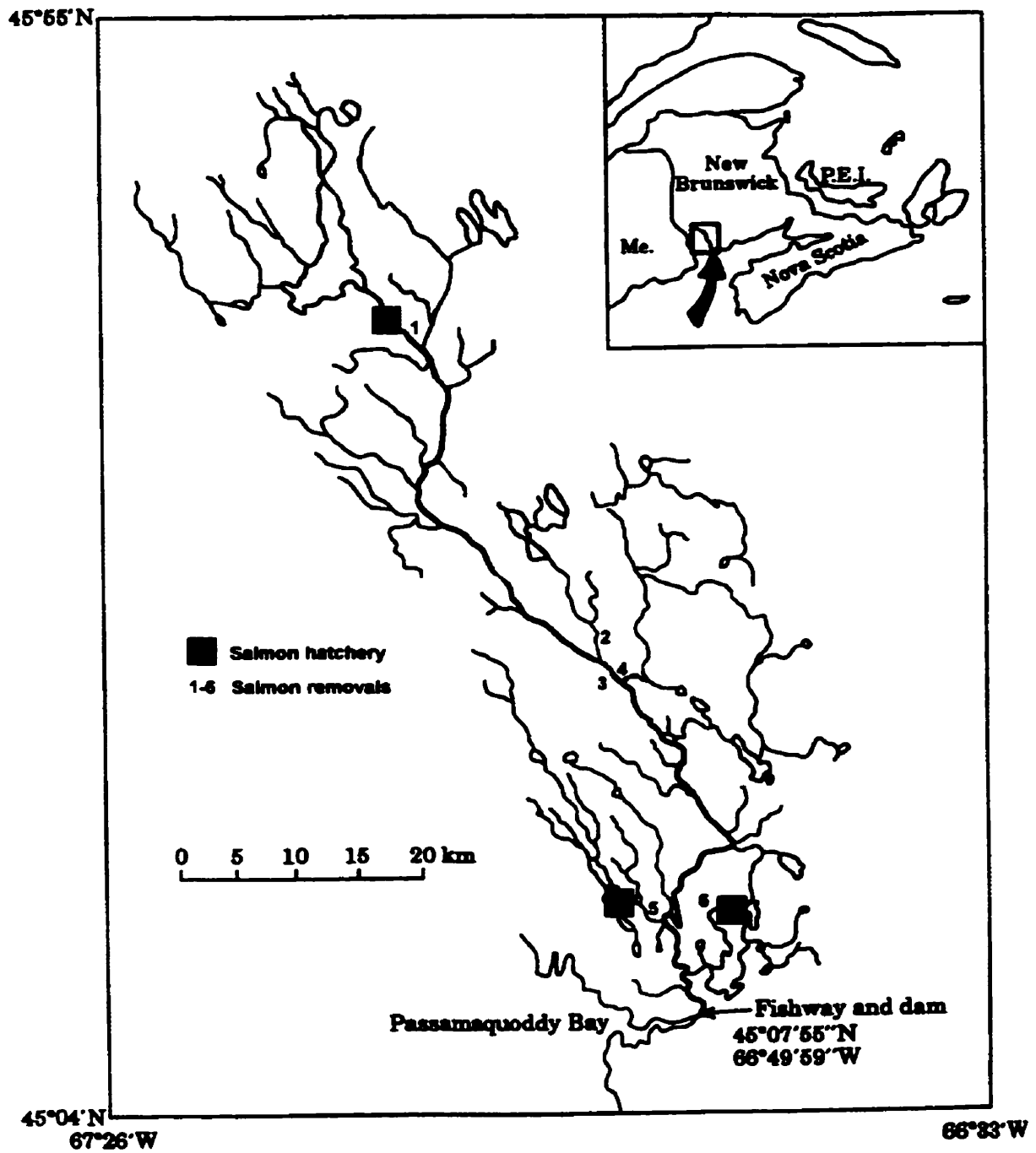


Figure 5. Wild juvenile Atlantic salmon removals from the Magaguadavic River, New Brunswick in 1997.

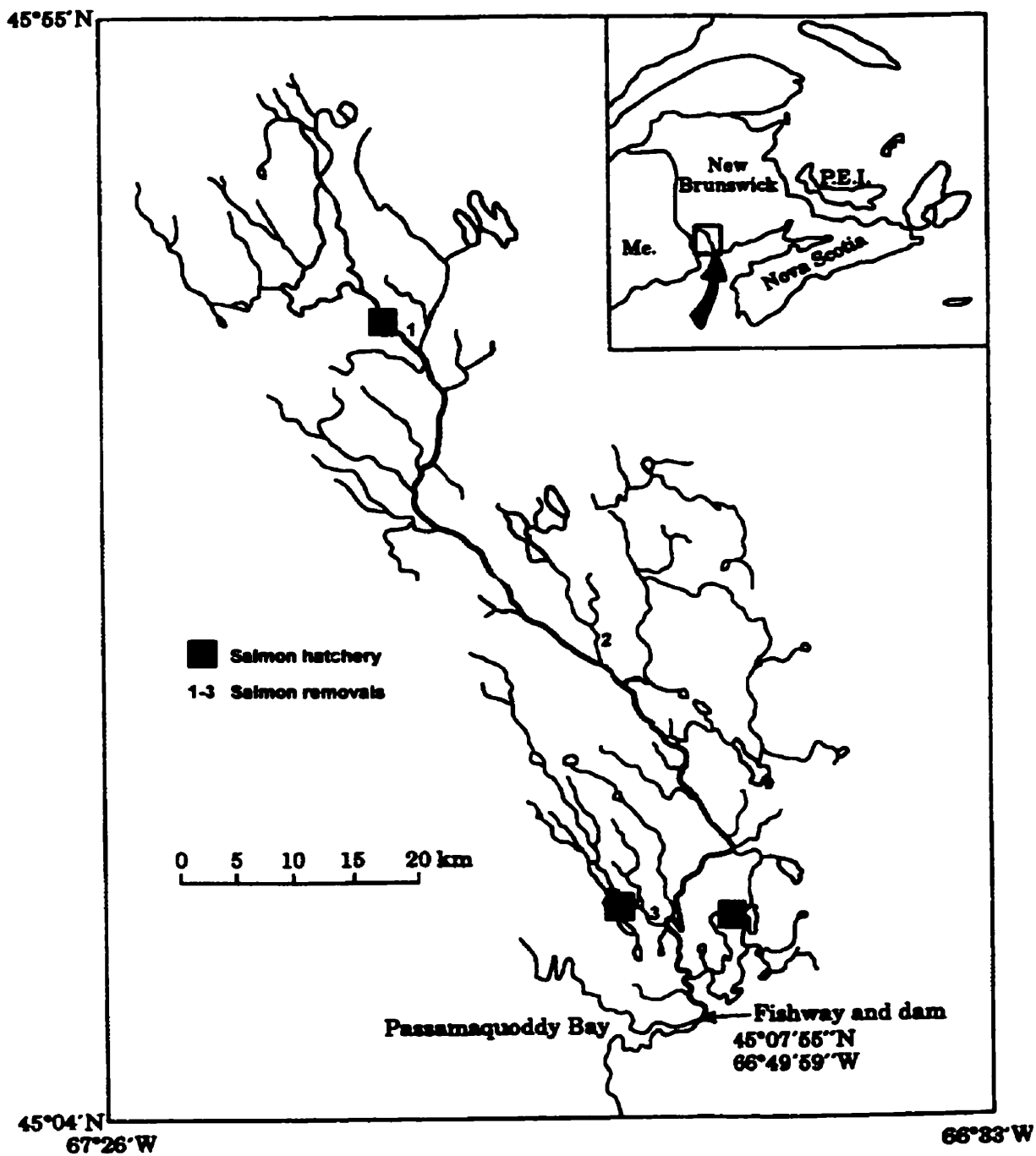
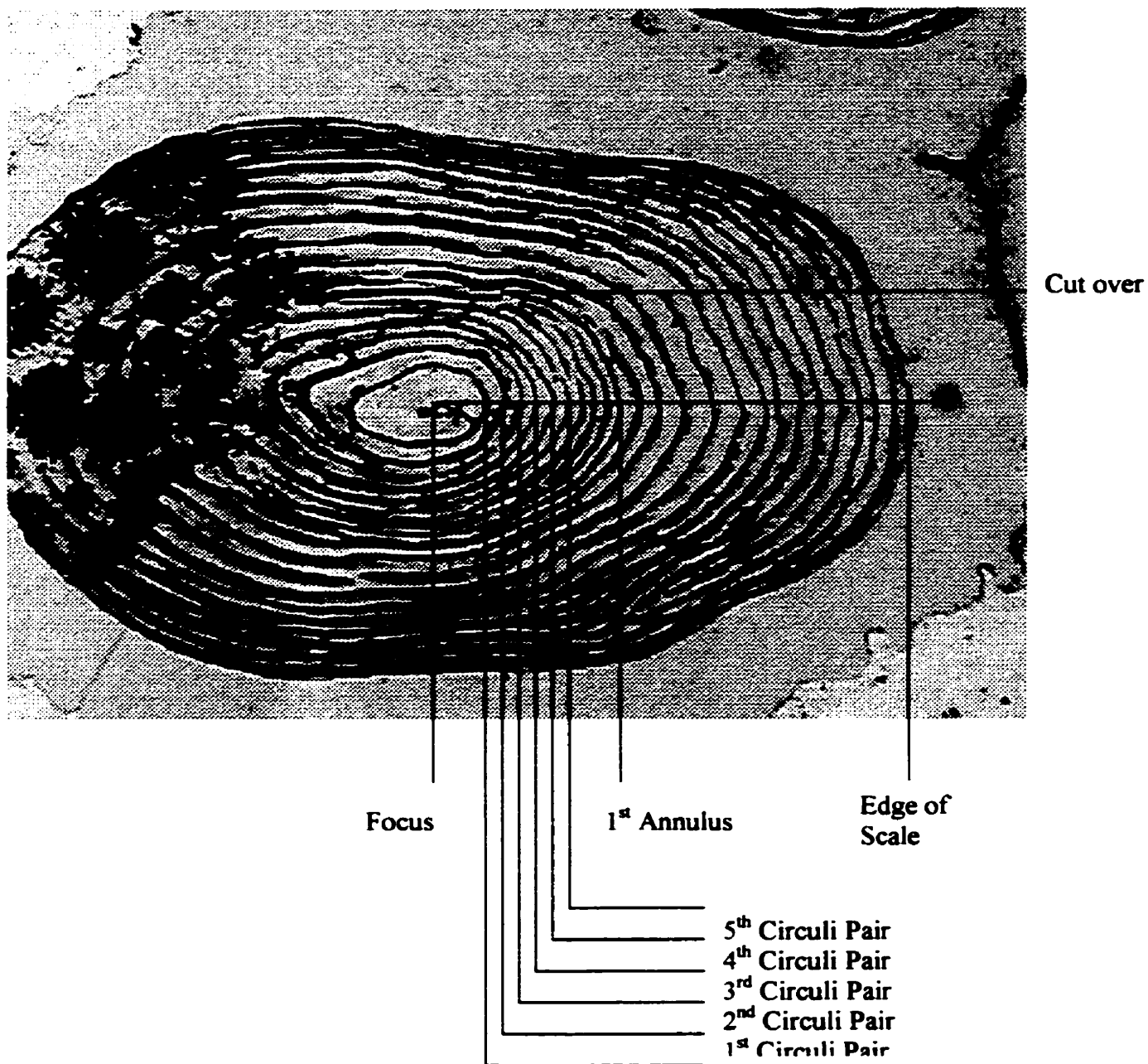


Figure 6. Wild juvenile Atlantic salmon removals from the Magaguadavic River, New Brunswick in 1998 (1 = Tomlinson Corner site; 2 = Kedron Stream site; 3 = Linton Stream site).



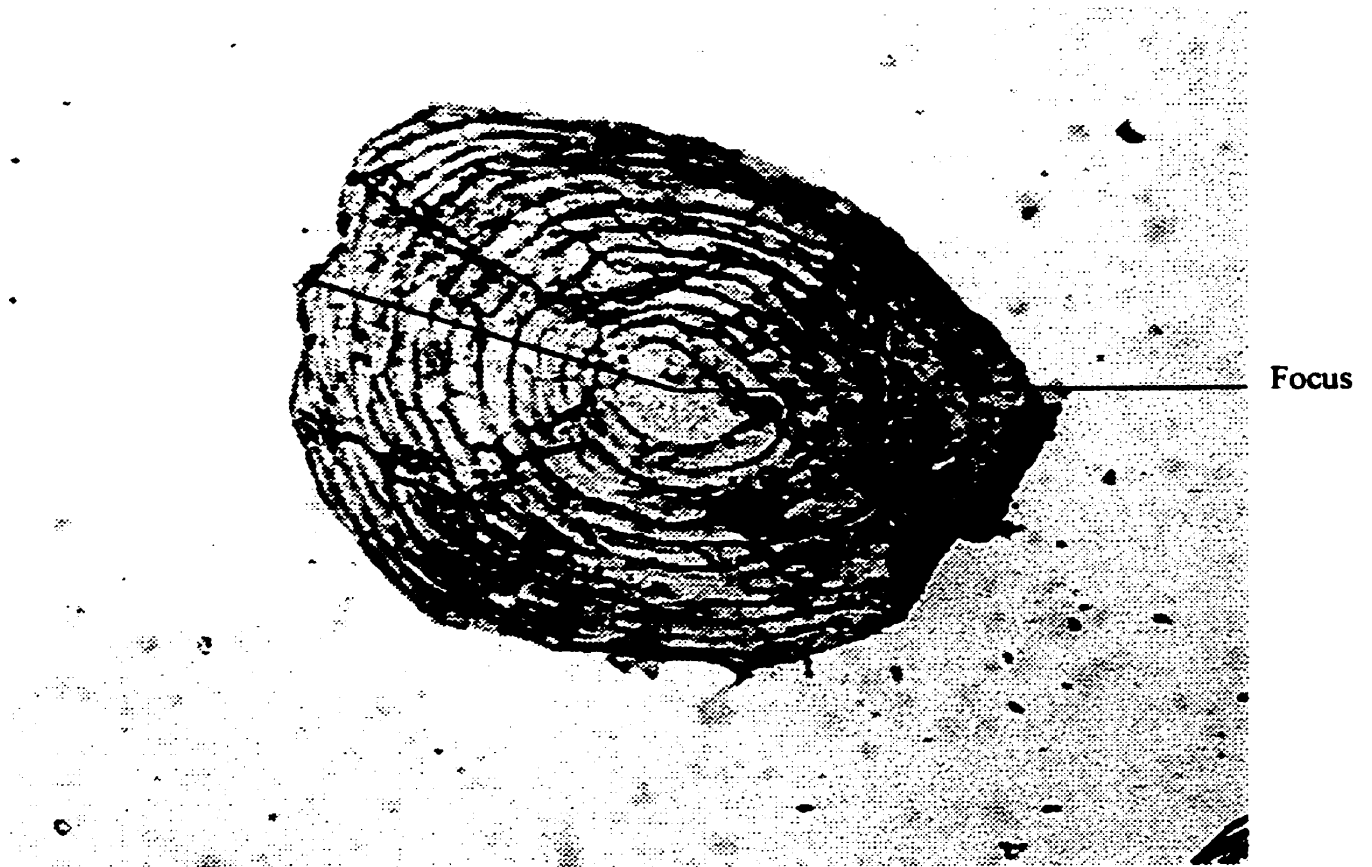
Figure 7. Scale from a 1-year-old Atlantic salmon taken from the Digdequash River, New Brunswick on 22 July 1998 and predicted to be of farmed origin by the discriminant function analysis. Reference line is shown on the longest axis of the scale from the center of the focus to the scales edge.



**Figure 8.** Scale from a 1-year-old Atlantic salmon from the Waweig River, New Brunswick captured on 23 July 1998 and predicted to be of native origin by the discriminant function analysis. Scale shows Circuli pairs used in the discriminant function as predictors of origin.



Figure 9. Scale taken from a 1-year-old Atlantic salmon on 27 January 1997 from Connors Brothers hatchery on the Magaguadavic River, N. B. Data from this scale was used in the formation of the “known origin farmed” sample for the discriminant function analysis. The area of the focus (inside of the first circuli) was generally larger for farmed salmon than for wild salmon; it was used as a predictor of farmed or native origin in the discriminate function analysis.

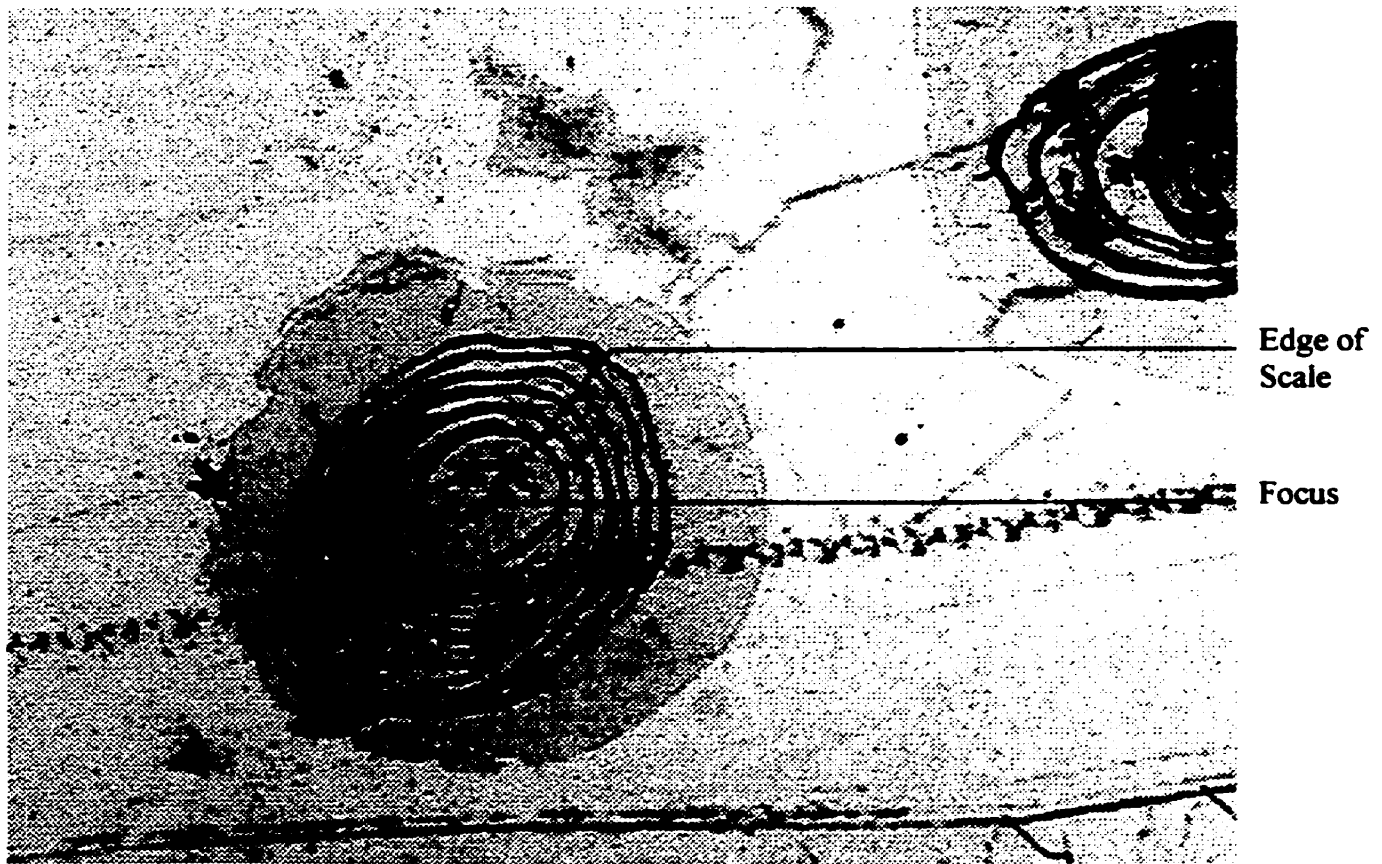


**Figure 10. Scale from a 0-year-old Atlantic salmon captured September 1997 at Tomlinson Corner, Magaguadavic River, N.B. This salmon was predicted as being of native origin by the discriminant function analysis. This scale has a degraded edge and would not be appropriate for evaluation by any method that required back-calculation of length.**





Figure 11. Scale taken from a 1-year-old Atlantic salmon on 27 January 1997 from the Stolt Sea Farm Inc. hatchery on the Magaguadavic River, N. B. Data from this scale was used in the formation of the "known farmed origin" sample for the discriminant function analysis. Note the large and consistent distance between the first five circuli pairs.



**Figure 12. Scale taken from a 0-year-old Atlantic salmon from Tomlinson Corner, Magaguadavic River. This salmon was predicted to be of farmed origin by the discriminant function analysis. This is an example of the minimum amount of circuli formation that a salmon may have and still be evaluated to natal origin by this discriminate function analysis procedure.**



Figure 13. Scale from a 0-year-old Atlantic salmon captured 21 July 1998 in Linton Stream, Magaguadavic River N. B. This salmon was predicted as being of farmed natal origin by the discriminant function analysis. The circuli patterns indicate that this juvenile has undergone rapid, consistent growth.

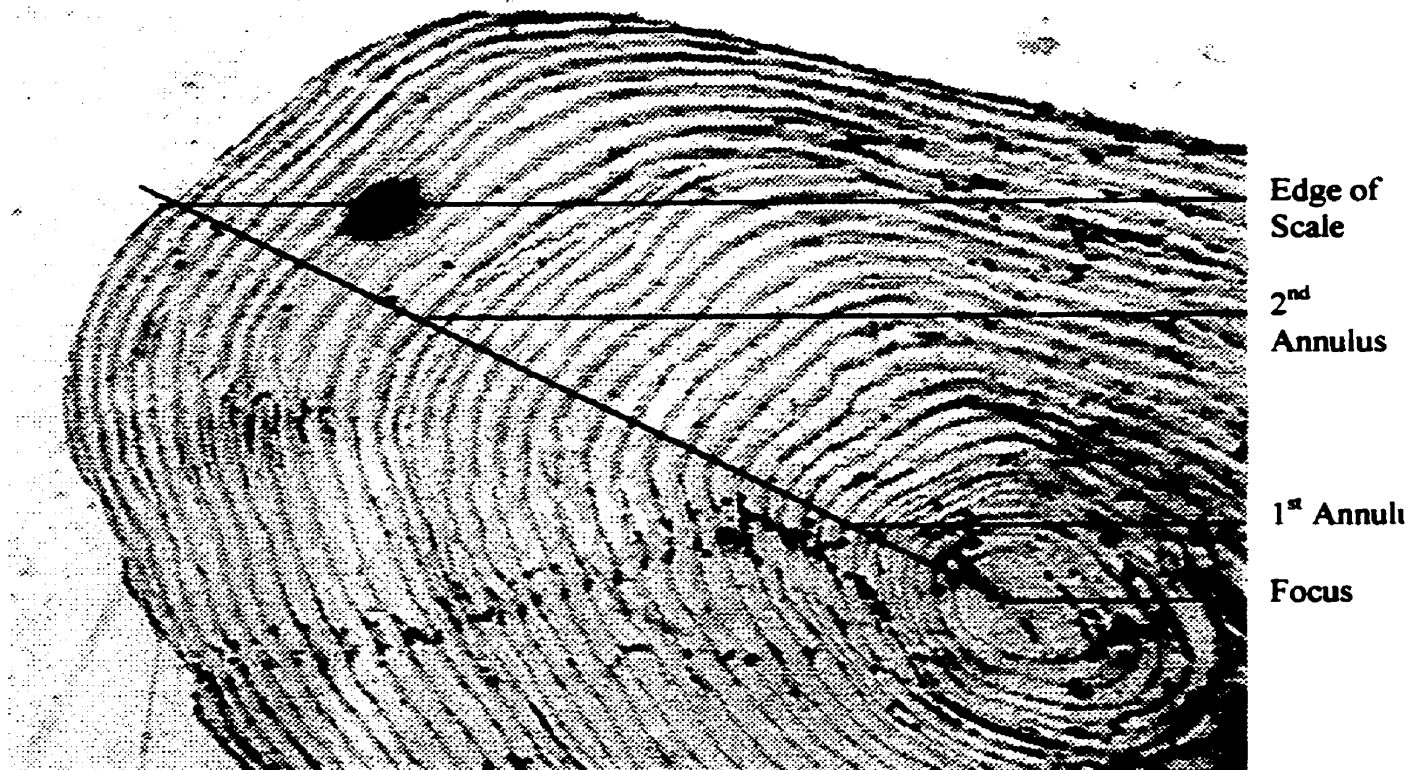


Figure 14. Scale from a 2-year-old Atlantic salmon captured in the lower Trout Brook of the Magaguadavic River N. B. Characteristics from this scale were used to compute the “known origin native” sample for the discriminant function analysis. At the second annulus there has been some adsorption of the scale reducing the number of the winter bands.

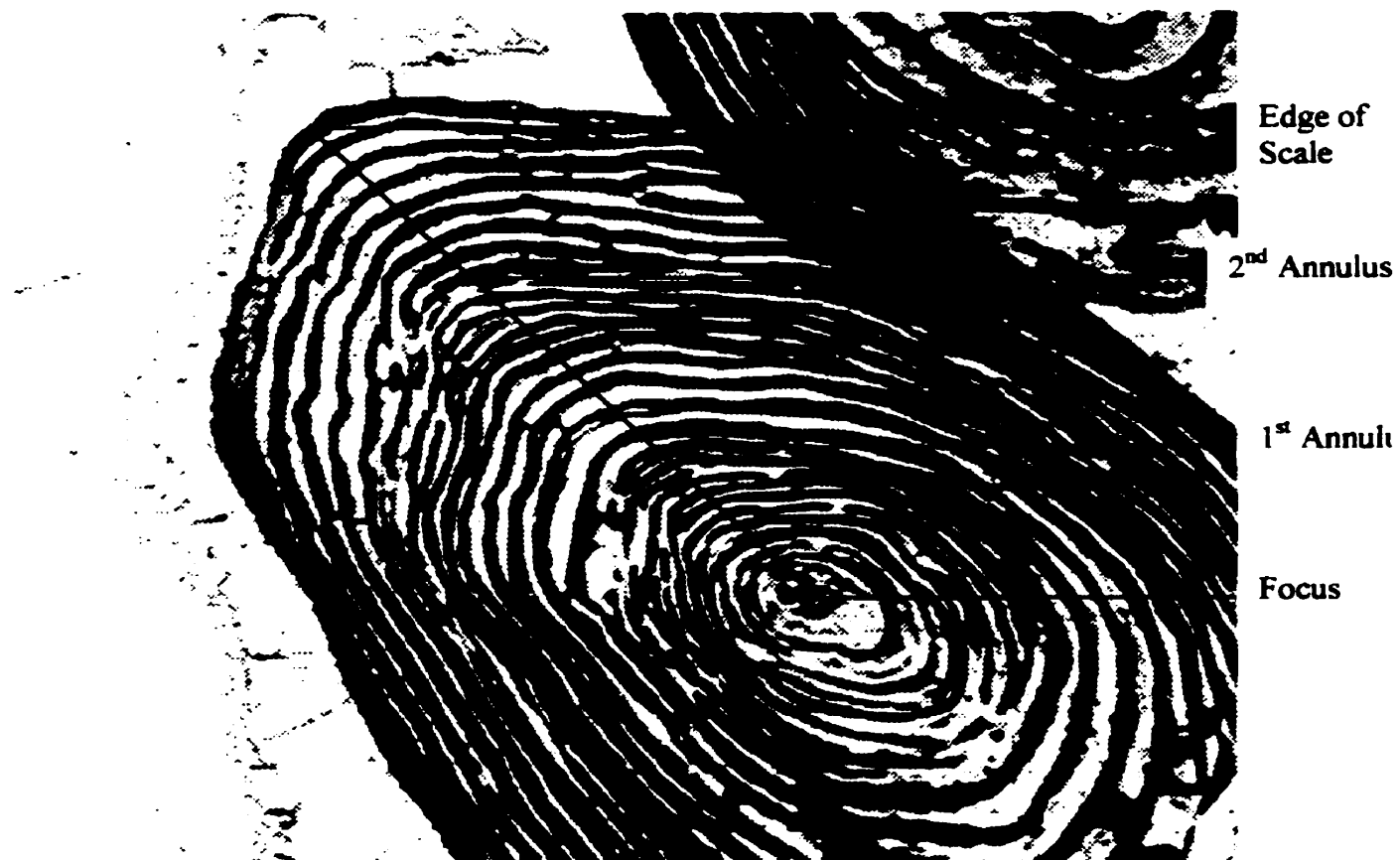


Figure 15. Scale taken from a 2-year-old Atlantic salmon collected on 19 August 1998, from the Kedron Stream of the Magaguadavic River N. B. This salmon was predicted native by the discriminant function analysis. Note the close spacing of the first five circuli pairs.

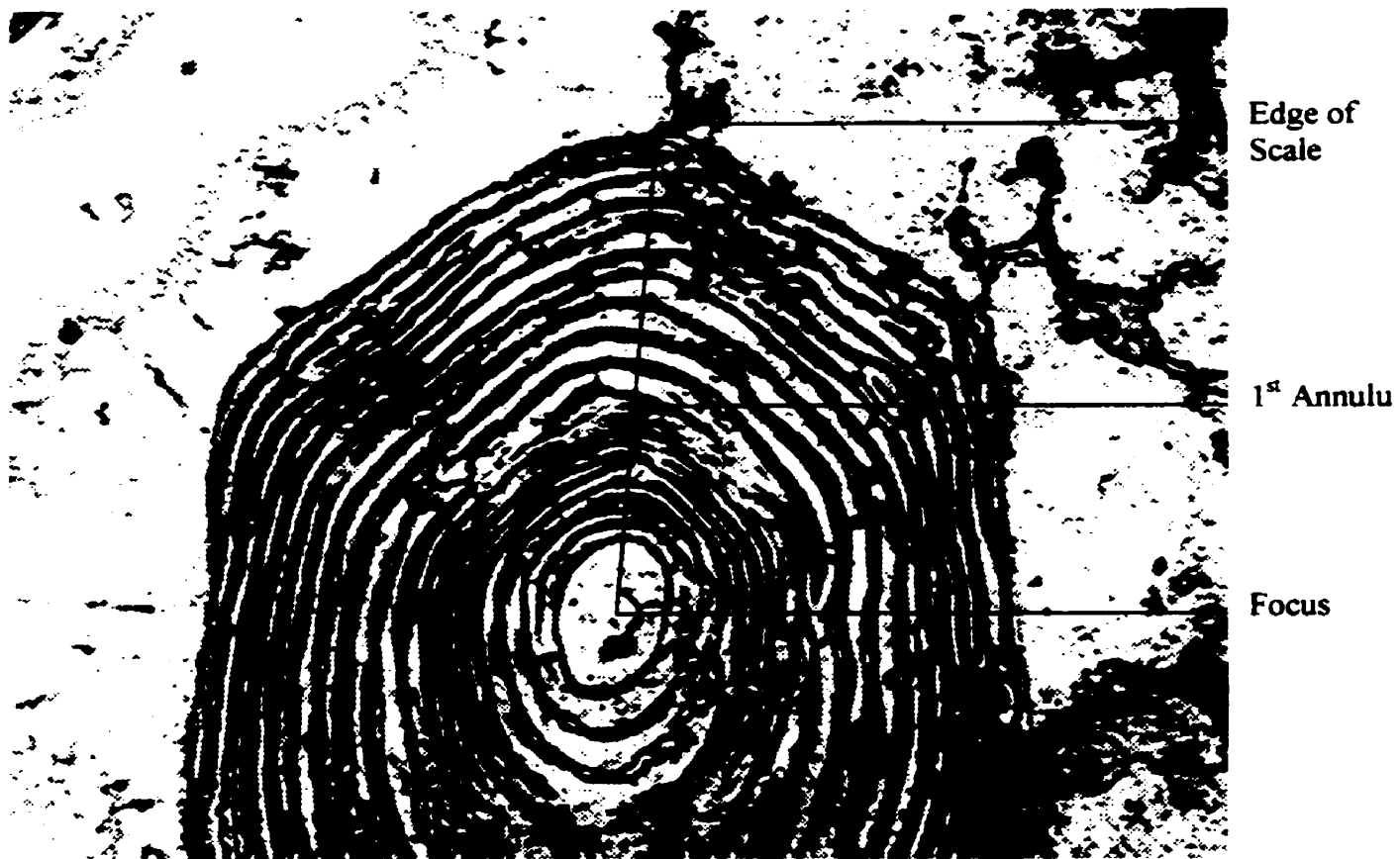


Figure 16. Scale from a 1-year-old Atlantic salmon taken from the Digdequash River, New Brunswick on 20 August 1998 and predicted to be of native origin by the discriminant function analysis. Note the inconsistent spacing of the first five circuli.



Figure 17. Scale from a 1-year-old Atlantic salmon captured on 27 September 1998 in the Linton Stream, Magaguadavic River, N. B. This salmon was predicted as being of native origin by the discriminant function analysis. The large amount of growth in the 1+ year may be attributed to the high temperature of the Linton stream in 1998.

Figure 18. Scale from a 2-year-old Atlantic salmon captured in the Dennis Stream, New Brunswick on 23 July 1998 and used to partly form the "known native origin" group for the discriminant function.







Edge of Scale

1<sup>st</sup> Annulus

Focus

Figure 19. Scale from a 1-year-old Atlantic salmon captured in the Waweig River, New Brunswick on 23 July 1998 and predicted to be of farmed origin by the discriminant function analysis. Note the consistent circuli formation.

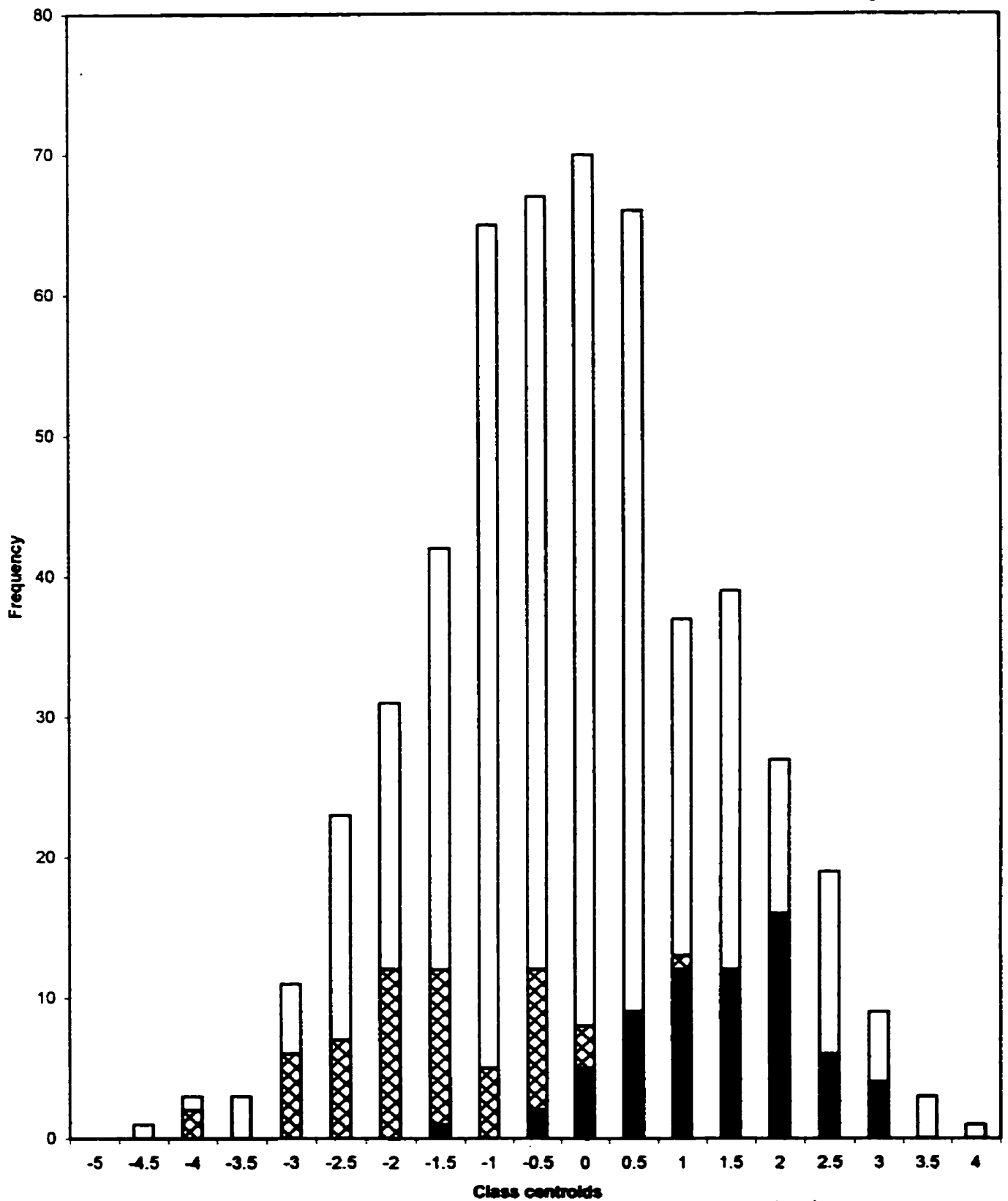


Figure 20. Distribution of known native (cross-hatched) farmed (black) and unknown origin (white) juvenile Atlantic salmon from the Magaguadavic River (1996-1998); as determined by a discriminant function analysis.

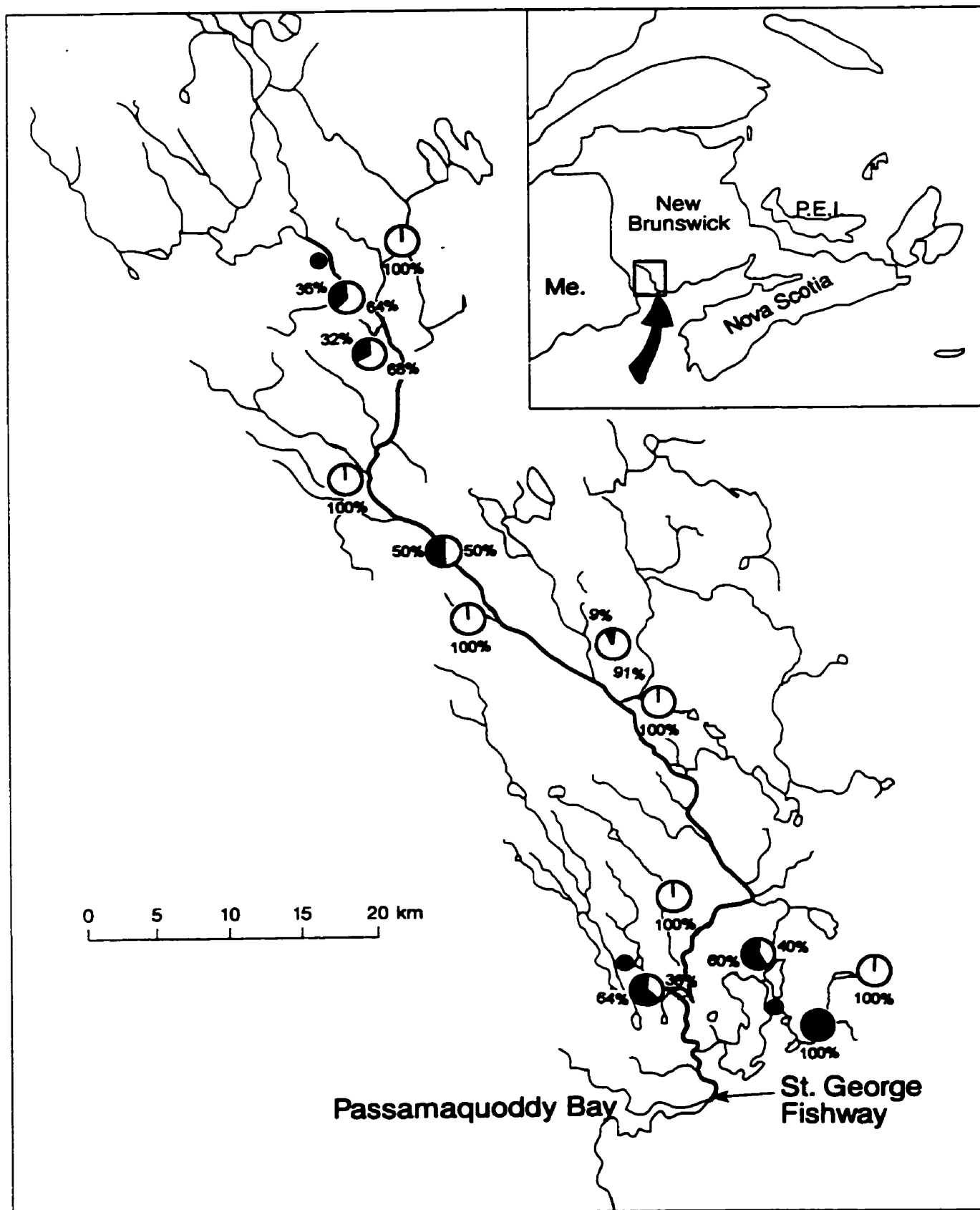


Figure 21. Percentage of farmed (black) and native (white) juvenile Atlantic salmon in collections from the Magaguadavic River, New Brunswick, 1996.

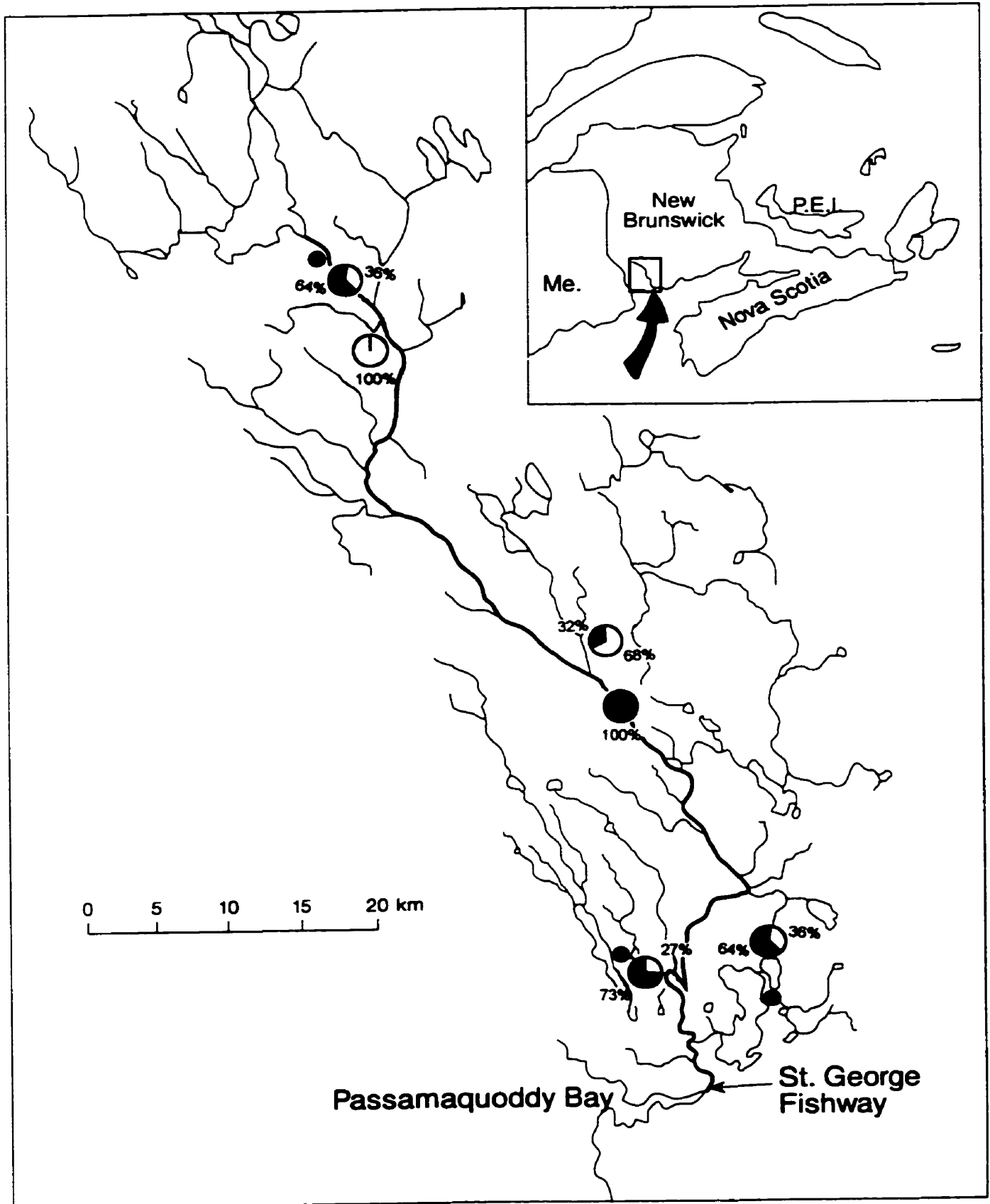


Figure 22. Percentage of farmed (black) and native (white) juvenile Atlantic salmon in collections from the Magaguadavic River, New Brunswick, 1997.

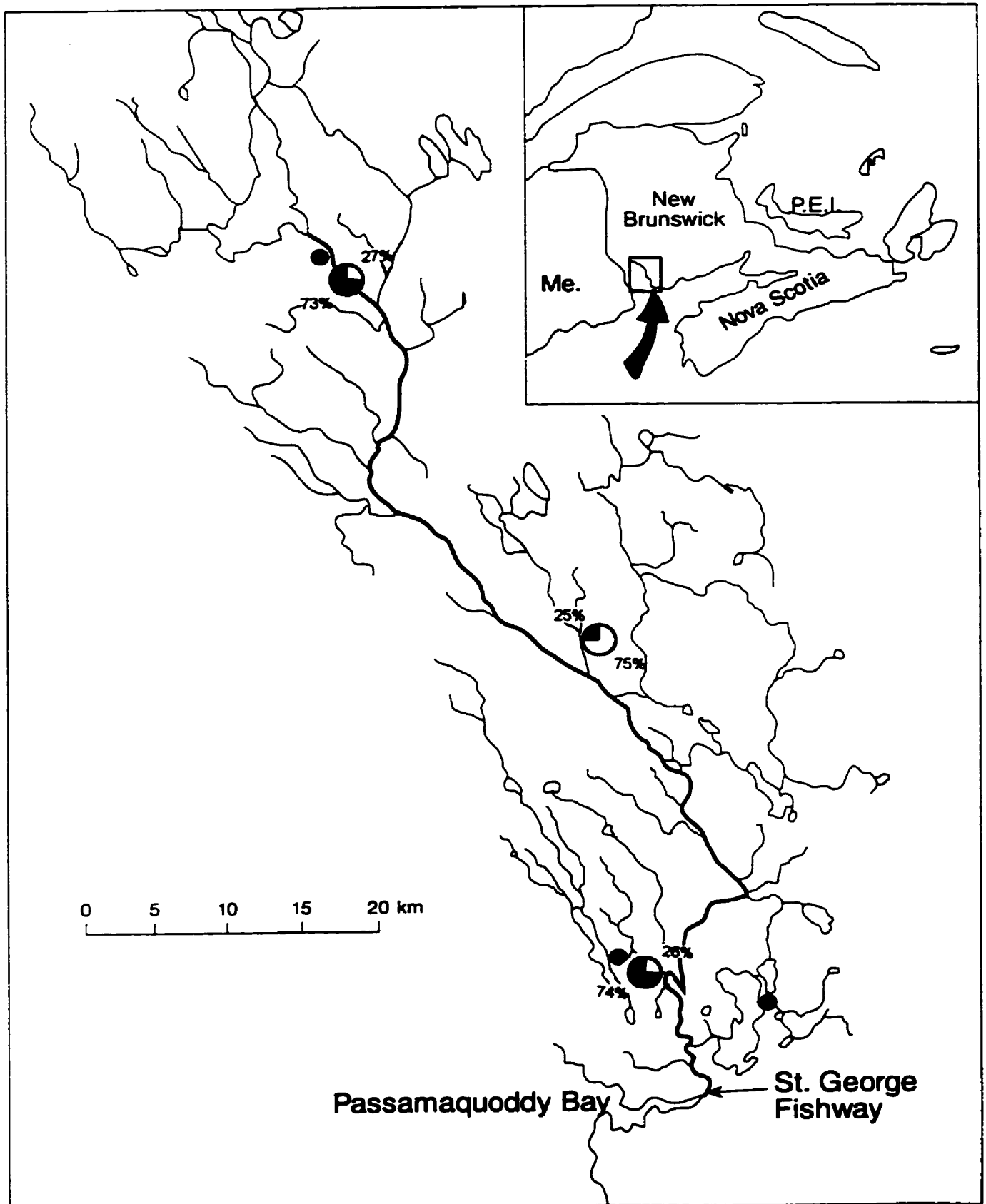


Figure 23. Percentage of farmed (black) and native (white) juvenile Atlantic salmon in collections from the Magaguadavic River, New Brunswick, 1998.

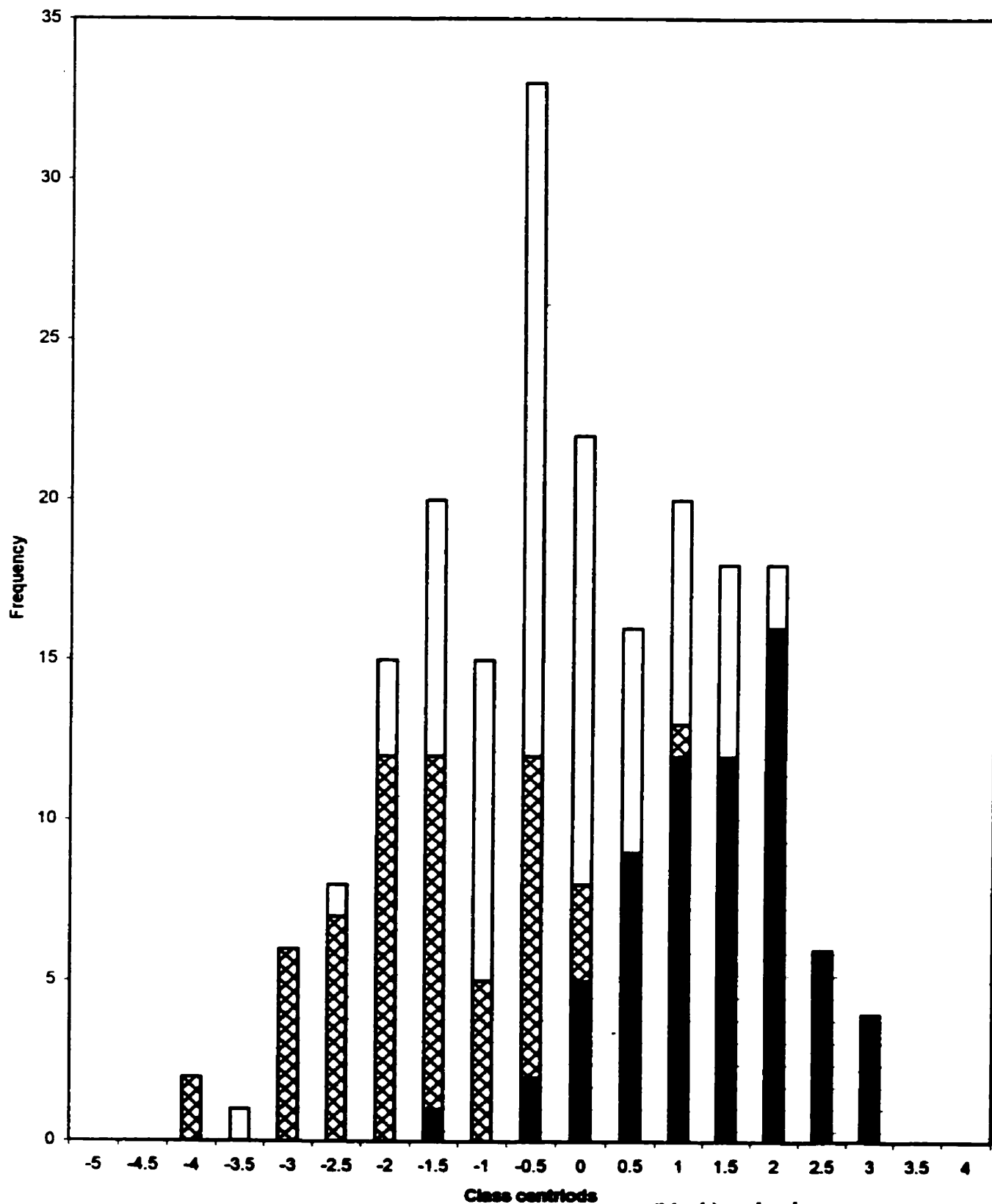


Figure 24. Distribution of known native (cross-hatched) farmed (black) and unknown origin (white) juvenile Atlantic salmon from the Digdequash River, 1998; as determined by a discriminant function analysis.

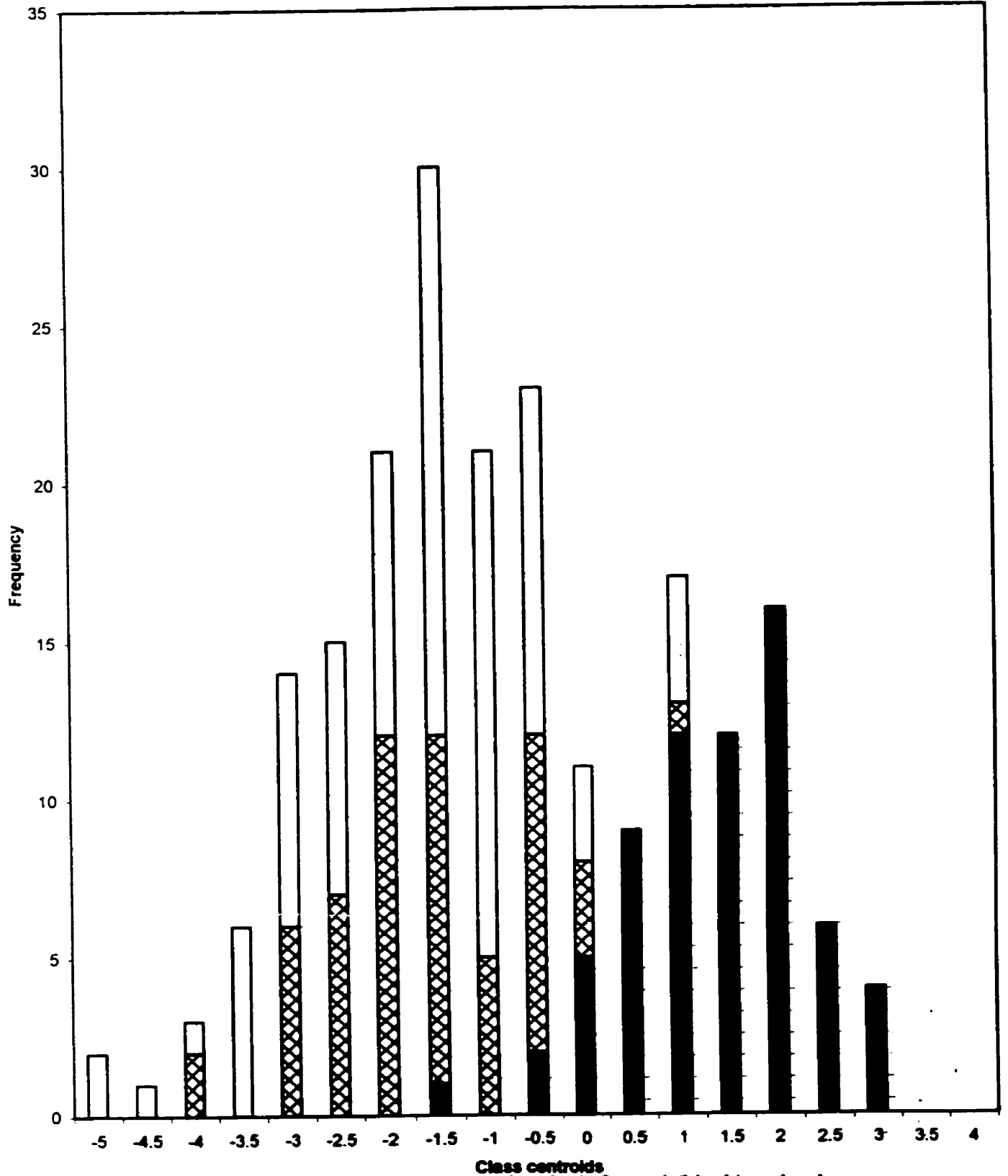


Figure 25. Distribution of known native (cross-hatched) farmed (black) and unknown origin (white) juvenile Atlantic salmon from the Waweig River, 1998; as determined by a discriminant function analysis.

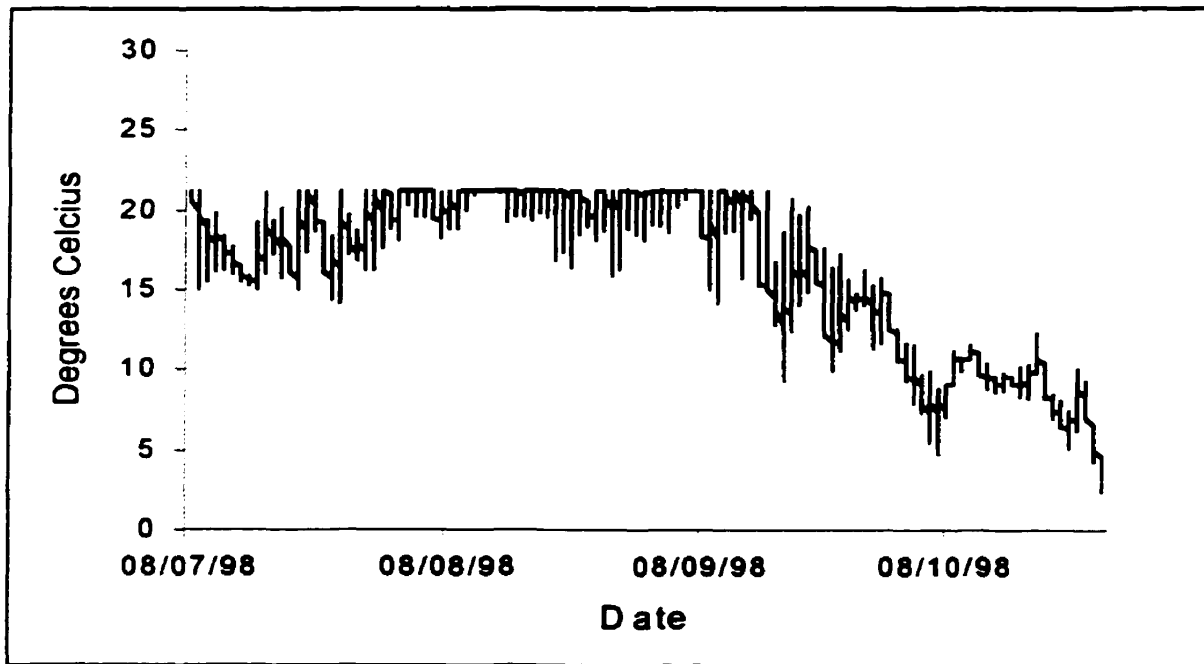


Figure 26. Temperature profile for the Kedron Stream, Magaguadavic River, New Brunswick in 1998. The temperature recorder's maximum temperature (21.5 degrees celcius) was reached from late July to mid-september resulting in a plateaued reading.



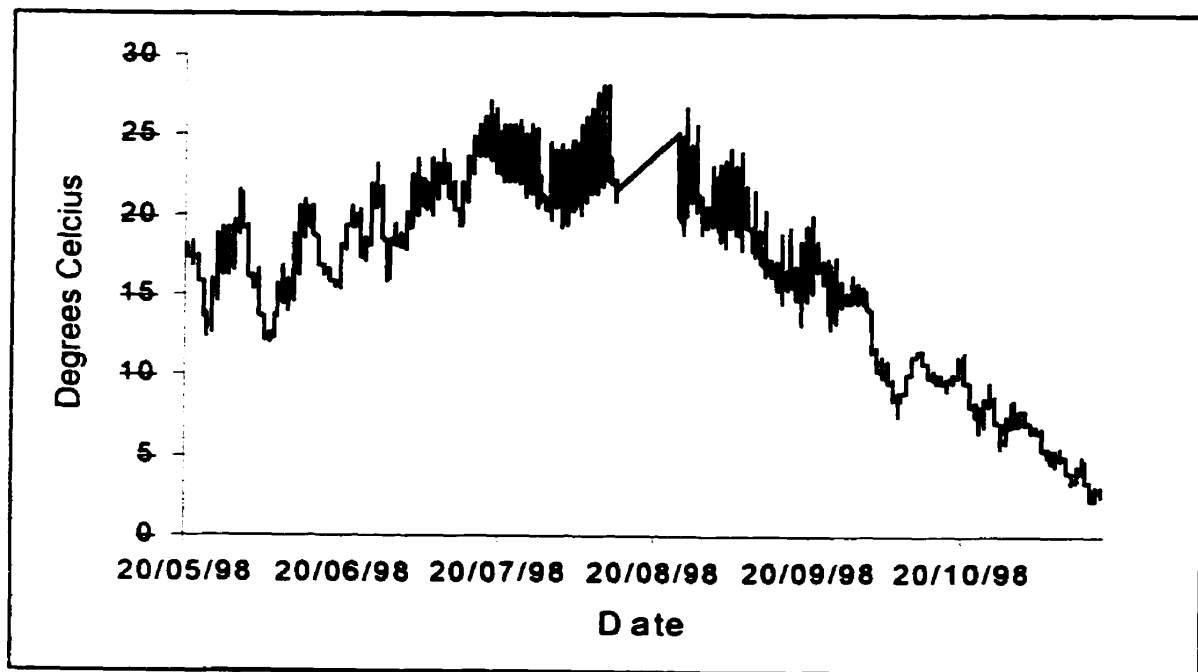


Figure 27. Temperature profile from the Digdequash River, New Brunswick, 1998.

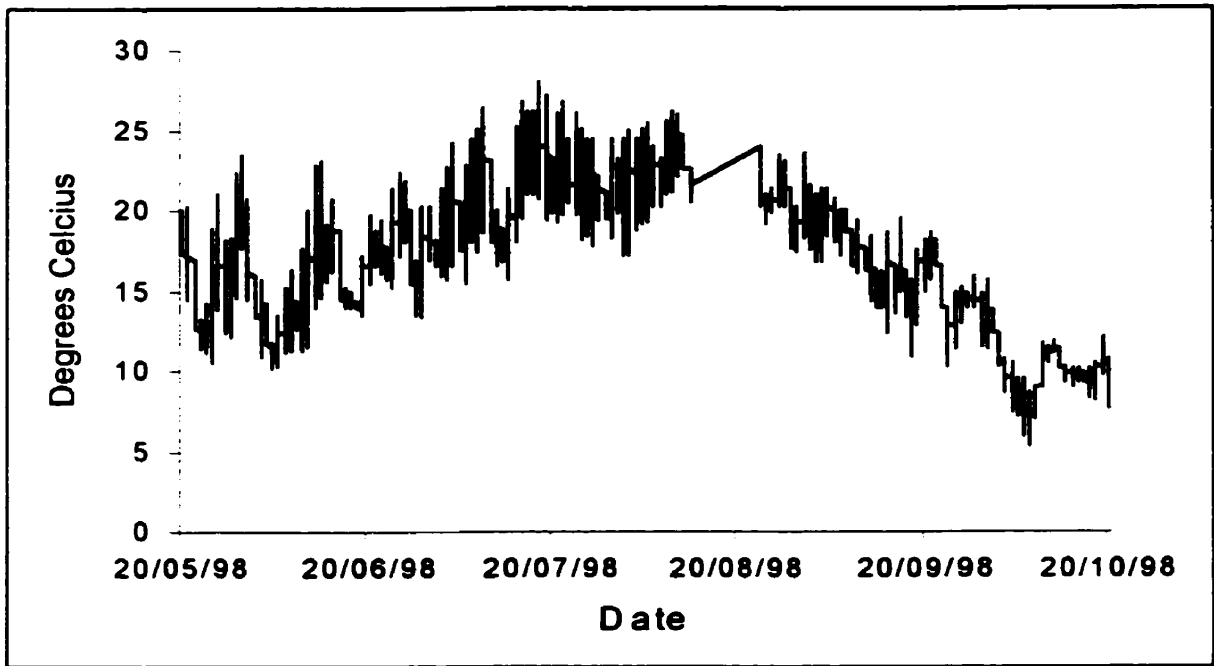


Figure 28. Temperature profile from the Waweig River, New Brunswick, 1998.

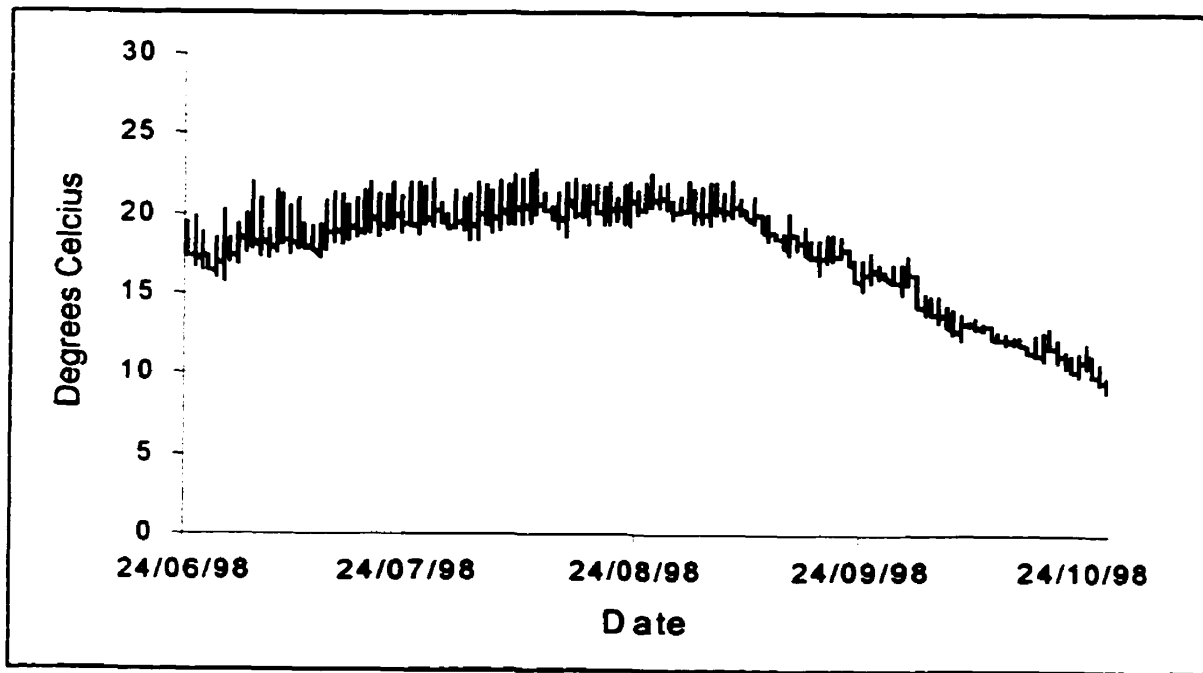


Figure 29. Temperature profile from the Linton Stream of the Magaguadavic River, New Brunswick in 1998.

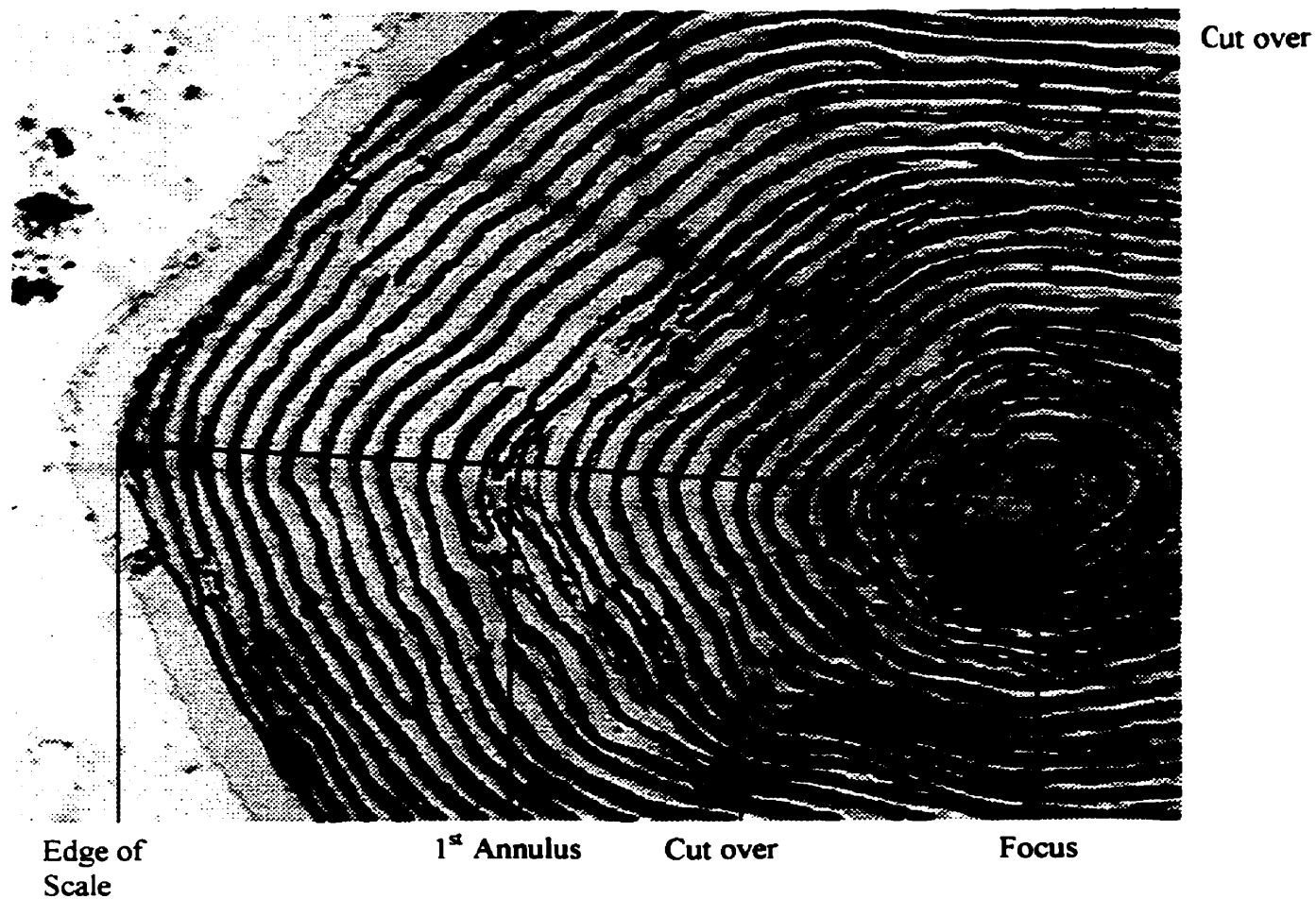


Figure 30. Scale from a 1-year-old Atlantic salmon collected 15 September 1998 from the Kedron Stream of the Magaguadavic River N. B. This fish was predicted farmed origin by the discriminant function analysis. Because this fish was captured approximately 40 km from a hatchery and up a side stream, it is more likely that it was an improperly marked stocked fingerling from stocking that occurred in the Kedron Stream in 1997.

## Appendix I

---

Groups of salmon	Definition
Wild	Salmon captured in the wild, regardless of natal history or parentage.
Farmed	Salmon born in culture for use in the commercial Atlantic salmon aquaculture industry.
Native	River born salmon regardless of whether their parents were river born or born in culture.
Stocked	Wild salmon that were born in captivity and intentionally released into their wild environment to supplement populations.
Juvenile escapees	Wild salmon that were born in captivity and escaped into the wild as juveniles.
Adult escapees	Wild salmon born in captivity that escaped into the wild from marine grow-out sites as adults.
Cultured juveniles	Juvenile salmon in captivity.

---