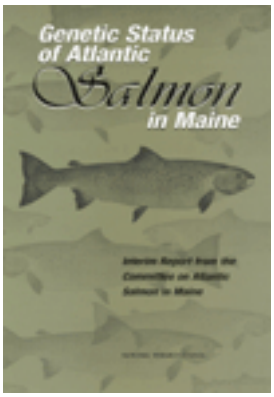

Genetic Status of Atlantic Salmon in Maine: Interim Report

Committee on Atlantic Salmon in Maine, Board on Environmental Studies and Toxicology,Ocean Studies Board,National Research Council

ISBN: 0-309-51052-X, 76 pages, 6 x 9, (2002)

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Interim Report from the Committee on Atlantic Salmon in Maine

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Board on Environmental Studies and Toxicology

Ocean Studies Board

Division on Earth and Life Studies

National Research Council

NATIONAL ACADEMY PRESS
Washington, D.C.

NATIONAL ACADEMY PRESS 2101 Constitution Ave., N.W. Washington, D.C. 20418

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This project was supported by Grant No. 01-0008 between the National Academy of Sciences and the National Fish and Wildlife Foundation. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the organizations or agencies that provided support for this project.

International Standard Book Number: 0-309-08311-7

Cover: design by Van Nguyen; salmon illustration by Walter H. Rich
from *Fishes of New England: The Salmons*, plate 4

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National Academy Press
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Acknowledgment of Review Participants

This report has been reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise, in accordance with procedures approved by the NRC's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making its published report as sound as possible and to ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We wish to thank the following individuals for their review of this report:

Edward Baum, Atlantic Salmon Unlimited
Roger Doyle, Genetic Computation Ltd.
William Hershberger, National Center for Cool and Cold Water Aquaculture
James Kitchell, University of Wisconsin
Irving Kornfield, University of Maine
Martha Mather, University of Massachusetts-Amherst
Nancy Targett, University of Delaware
Fred Utter, University of Washington
David Wake, University of California, Berkeley

Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations nor did they see the final draft of the report before its release. The review of this report was overseen by John Dowling, Harvard University. Appointed by the National Research Council, he was responsible for making certain that an independent examination of this report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with the authoring committee and the institution.

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Summary

Atlantic salmon in Maine, once abundant but now seriously depleted, were listed as endangered under the federal Endangered Species Act (ESA) in November 2000. The listing covers the wild fish in eight Maine rivers as a single "distinct population segment." The controversy in Maine that accompanied the listing led Congress to request the National Research Council's (NRC's) advice on the science relevant to understanding and reversing the declines in Maine's salmon populations. The charge to the NRC's Committee on Atlantic Salmon in Maine included an interim report focusing on the genetic makeup of Maine Atlantic salmon populations. This is the interim report.

Understanding the genetic makeup of Maine's salmon is important for recovery efforts, because the degree to which populations in Maine differ from adjacent populations in Canada and the degree to which populations in different Maine rivers and tributaries differ from each other affect the choice of recovery options that are most likely to be effective. This report focuses only on questions of genetic distinctiveness. The committee's final report will address the broader issues, such as the factors that have caused Maine's salmon populations to decline and the options for helping them to recover.

SALMON BIOLOGY

Naturally reproducing populations of Atlantic salmon occur in rivers and streams from southwestern Maine to northwestern Europe. Historically, they were found in the Hudson River in New York and north and east to the Cana-

dian border but today are found only in Maine, from the lower Kennebec River to the Canadian border. The populations have declined drastically, from perhaps half a million adults returning to U.S. rivers each year in the early 1800s to about 1,000 in 2000.

Salmon spawn in fresh water, where the young hatch and grow for a year or 2 before migrating to sea. At sea, they grow faster in the rich marine environment and then return to the rivers where they hatched (called natal streams) to spawn. Most fish die after spawning, but some return to the ocean, and some of those return to spawn again. Adults return to their natal streams; only about 2% stray to other (usually nearby) streams.

The occasional straying is probably important evolutionarily, because it allows recolonization of a stream if the local population dies out and provides for small infusions of new genetic material for evolutionary adaptation to changing conditions. Their homing provides an opportunity for the salmon to adapt to environmental conditions in their natal streams. This complex life-history pattern makes salmon vulnerable to environmental disruptions both at sea and in fresh water. It also complicates the understanding of the genetic makeup of salmon populations because of the relationship between local adaptations and exchange of genetic material through occasional straying.

HATCHERIES AND AQUACULTURE

Augmentation of wild populations of Maine salmon with hatchery releases began in the early 1870s. At first, young fish were obtained from Lake Ontario, and then the Craig Brook Hatchery, using eggs from Penobscot River fish in Maine, was the stocking source. By the 1920s, Canadian eggs were being used, followed in the 1940s by eggs from the Machias, Penobscot, and Dennys rivers of Maine. In the 1950s and 1960s some eggs of Canadian origin were used again, but by the late 1960s, eggs from Maine's Machias, Narraguagus, and Penobscot rivers were used. Fish reared in hatcheries derived from Penobscot River fish were used until late 1991, when the practice of river-specific stocking was adopted. The protocol used since involves catching young, actively feeding fish (parr) in the river, rearing them to maturity in the hatchery, mating them, and releasing the resulting fry into the rivers before they start to feed.

In addition to stocking, which at least until 1992 added to rivers many fish (and eggs) whose genotypes did not reflect adaptation to the local environment, aquaculture (farming) of Atlantic salmon began in Maine in the 1980s, the first

fish for market being produced in 1987. Derived in part from European Atlantic salmon, the genetic strains used for fish farming are even more different from native strains than hatchery strains. Farm fish escape at all life stages, despite the efforts of producers to prevent escapes. In some years and in some rivers, more escaped farm fish return to spawn than wild fish. The impact of escapees on the genetics of wild populations is not well documented in Maine, but both hatchery- and pen-reared fish compete poorly in rivers with wild fish in other areas that have been studied. However, because there are so many escaped farm fish compared with wild fish in some rivers, some impact is likely to have occurred, especially as farm production has increased in recent years.

The addition of so many nonwild genotypes from hatcheries and possibly from aquaculture escapees has led some to conclude that the fish returning to spawn in Maine's rivers could not possibly represent anything more than some nonnative mix of genotypes from Europe, Canada, and Maine. If that were true, then options for conservation might be considerably different from those that might be undertaken if the wild fish in Maine were distinct, and that is why it is important to understand the genetic makeup of the wild salmon populations in Maine.

THE DATA ON GENETICS OF MAINE SALMON

The committee's focus in this interim report is on assessing how Maine salmon populations differ from other Atlantic salmon populations and among themselves. The committee has addressed the question at three levels. First, are North American Atlantic salmon genetically different from European salmon? Second, are Maine salmon distinct from Canadian salmon? Third, to what degree are salmon populations in the eight Maine rivers mentioned in the ESA listing distinct from each other?

Much of the evidence on genetic distinctiveness is based on laboratory analyses of variations in the gene products (proteins) and in the genetic material (DNA) itself. The preliminary evidence indicated distinctiveness at all three levels, and that indication led to the ESA listing. However, the evidence has been questioned on statistical, methodological, biological, and other grounds, and so it bears close evaluation.

The committee evaluated the original evidence, including technical reports, as well as newly published information. It reviewed earlier studies and studies of similar situations involving other locations and some other species of fishes

in the salmon family and considered the questions raised about the evidence on Maine salmon. In addition, the committee considered the effect that overlapping generations[1] of salmon might have on the evidence.

The evidence on distinctiveness of Maine salmon includes statistical studies on a variety of protein and DNA markers. The statistical significance of the results is so strong and the departures from random expectations are so large that the committee judged the results to be persuasive. Many appropriate questions have been raised about the evidence, and the most recent studies have benefited from criticisms of earlier work. Those criticisms could still be used to improve future work, but the general conclusions are so strongly supported by the evidence that they are not invalidated by imperfections in the data collections or analyses.

The committee concludes that North American Atlantic salmon are clearly distinct genetically from European salmon. In addition, despite the extensive additions of nonnative hatchery and aquaculture genotypes to Maine's rivers, the evidence is surprisingly strong that the wild salmon in Maine are genetically distinct from Canadian salmon. Furthermore, there is considerable genetic divergence among populations in the eight Maine rivers where wild salmon are found.

The heavy stocking of salmon in Maine's rivers and streams has included periods of heavy Canadian stocking, interspersed with strictly Maine stocking. Exactly how much Canadian genetic material has infiltrated Maine salmon populations is impossible to judge at this date.

It is thus appropriate to ask whether wild salmon in Maine reflect only (or mainly) the result of decades of hatchery stocking. That seems unlikely, because if that were so, Maine salmon should be more similar to Canadian salmon than they are. In addition, if their genetic makeup were largely due to stocking of nonlocal salmon broadly across Maine's rivers, salmon populations within Maine would be genetically much more similar than they are. A related question is whether the genetic differences among the fish in the various Maine streams reflect natural processes that occur in watersheds that are connected in networks. More specifically, the issue concerns the relative importance of natural selection over long periods, which influenced the differentiation of Maine's original salmon populations, versus recent genetic drift (sampling effects) caused by small populations. This question cannot be an-

[1]Before progeny hatched in a particular year can reproduce, progeny hatched in earlier years will reproduce. Thus, the generations overlap.

swered at present, but the pattern of genetic variation seen among Maine streams is similar to patterns seen elsewhere in salmon and their relatives where no stocking has occurred. Maine streams have salmon populations that are genetically as divergent from Canadian salmon populations and from each other as would be expected in natural salmon populations anywhere else in the Northern Hemisphere.

1
Introduction

BACKGROUND

Maine was once the home of abundant populations of wild Atlantic salmon (*Salmo salar*), but they have been declining since at least the middle of the nineteenth century (Baum 1997). Despite conservation efforts over the past 130 years or so, populations in Maine have continued to decline, and now they are seriously depleted in all the rivers that still retain natural runs. Only about 1,000 adults returned to Maine streams to spawn in 2000. The declines led to the listing of Atlantic salmon in eight Maine rivers (Cove Brook, Dennys, Ducktrap, East Machias, Machias, Narraguagus, Pleasant, and Sheepscot) as endangered under the federal Endangered Species Act (ESA) by the Fish and Wildlife Service (FWS) and the National Marine Fisheries Service (NMFS) (Figure 1). Those eight rivers together had only around 100 returning adults in 2000.

No one disputes the general seriousness of the declines, but many people in Maine claim that the populations are not wild and, therefore, oppose the ESA listing.[1] They argue that the fish are derived mainly from hatchery

[1]The term *wild* is used by the committee to mean populations of salmon that have been maintained by natural spawning for at least two full generations. The committee agrees with Baum (1997) that *pristine* salmon populations—populations that have always been wild with no human influences on their genetic makeup—almost surely do not exist in Maine. The term *natural* is used for salmon populations that are derived

FIGURE 1 USA Atlantic salmon rivers with active restoration/recovery programs in New England. *The eight DPS rivers in Maine listed as endangered under the ESA are (5) Dennys, (6) East Machias, (7) Machias, (8) Pleasant, (9) Narraguagus, (11a) Cove Brook, (12) Ducktrap, and (13) Sheepscot. Source: E. Baum, Atlantic Salmon Unlimited, unpublished material, 2001. Printed with permission of the author.

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from parents' reproduction in streams rather than stocking.

stocking and aquaculture escapes. If so, then appropriate measures to increase the number of salmon in Maine's rivers could be quite different from appropriate measures to increase wild salmon runs in those rivers. The controversy led Congress to mandate a study of Atlantic salmon in Maine by the National Research Council (NRC), including an interim report in time to help any recovery efforts (see Appendix A for committee's statement of task).

This is the interim report. The NRC's Committee on Atlantic Salmon in Maine hopes it will help to inform any future salmon-recovery efforts, whether they are undertaken under the ESA or otherwise, because if the populations are genetically indistinguishable, then it would be hard to justify recovery programs that treat the populations in different drainages separately, whereas if they are distinguishable, then such programs might be justifiable. The report focuses on the genetic characteristics of the wild populations in Maine, especially in the listed rivers.

THE LISTING OF SALMON UNDER THE ENDANGERED SPECIES ACT

The ESA of 1973 as amended, most recently by Public Law 100-478 in 1988, defines species as including "any subspecies of fish or wildlife or plants, and any distinct population segment of any species o[f] vertebrate fish or wildlife which interbreeds when mature" (Section 3{15}). The salmon in the eight Maine rivers—including "all naturally reproducing wild populations and those river-specific hatchery populations of Atlantic salmon having historical, river-specific characteristics found north of and including tributaries of the lower Kennebec River to, but not including, the mouth of the St. Croix River at the U.S.-Canada border"—were listed as an endangered distinct population segment (DPS) by FWS and NMFS ("the Services") on November 17, 2000 (DOI and DOC 2000). The science that underlies the ESA; the concept of species, including subspecies and DPSs; and the meaning of "endangered" under the ESA are discussed in considerable detail in two earlier NRC reports (NRC 1995, 1996).

In this interim report, the committee focuses only on the genetic makeup of natural populations of salmon in Maine and whether they are distinct from salmon populations elsewhere and from each other. The committee's final report will address broader issues, such as factors contributing to the decline of Maine's salmon populations and options for helping them to recover.

THE PRESENT STUDY AND REPORT ORGANIZATION

For this study, the committee met twice in Maine. At its first meeting in Bangor on June 12-14, 2001, the committee heard presentations from representatives of the state government of Maine, including Governor Angus King; from the Services; from the Atlantic Salmon Commission; and from a variety of industry, academic, environmental, and other private organizations and individuals. At its second meeting on September 20-22, 2001, members of the committee visited an Atlantic salmon farm and two blueberry farms in Washington County, a weir on the Pleasant River, and the federal hatchery at Craig Brook. The committee received additional briefings in Bangor. A complete list of the presenters and facilities visited is in Appendix B. The committee also considered an array of published literature and reports.

Section 2 of the report briefly reviews the biology and evolution of Atlantic salmon in Maine. Section 3 describes the current state of Atlantic salmon in Maine, including their stocking history and the aquaculture escapements that are relevant to the question of wild- population genetic makeup. Section 4 provides a description of the available data on genetic makeup and analyses of these data. Section 5 discusses data quality and related issues. Finally, Section 6 presents the committee's conclusion that the wild salmon populations in Maine are genetically distinct from salmon in Canada and elsewhere; furthermore, there is divergence even among populations within Maine. This pattern and degree of genetic variation among populations is consistent with the patterns observed in wild salmon populations elsewhere.

2
Biology and Evolution of Atlantic Salmon

NATURAL HISTORY OF ATLANTIC SALMON IN MAINE

Anadromy

Atlantic salmon, like their Pacific cousins, are anadromous: they begin their lives in fresh water, where the young grow to several inches in length, and then migrate to the sea, where they grow more rapidly and become sexually mature after 1, 2, or 3 years[1] (Baum 1997). Maine's Atlantic salmon exhibit two run timings that are in part influenced by genetic factors. "Early run" adults enter fresh water between May and mid-July, and "late-run" adults enter fresh water later in the summer. Some rivers have only early or late-runs, large rivers have both. Unlike their Pacific cousins, which always die after spawning, some Atlantic salmon survive spawning and return to sea, either soon after spawning or the following spring (1-6% according to Baum 1997). Some of these fish might spawn again.

Homing

When salmon return to rivers from the sea to spawn, they return to their natal stream (the stream where they hatched). Straying, or returning to an-

[1]Fish that return after 1 year are termed 1SW (one sea-winter) fish; 2SW and 3SW mean fish that spend two and three winters at sea, respectively. Fish that spend multiple winters at sea are called multi-sea-winter (MSW) fish.

other stream, occurs, but only occasionally. For example, Penobscot River salmon show more than 98% fidelity to the home stream (Baum 1997).

Distribution

Atlantic salmon are distributed from about 40° N, northward, on both sides of the Atlantic Ocean. In Europe, wild salmon breed from western Russia to Iceland and south to northern Spain; on the western side, wild breeding populations are found from Labrador to Maine. These distributions have been influenced by geological changes, including ice ages (MacCrimmon and Gots 1979). Populations in the United States probably date from the end of the last ice age 10,000 years ago. Atlantic salmon were probably present in all watersheds from the Hudson River in New York north to the St. Croix River, the Canadian border (Kendall 1935). Atlantic salmon once occupied 34 rivers and streams in Maine (Beland 1984). Today, wild Atlantic salmon populations in the United States are found only in Maine,[2] from the lower Kennebec River in the southwest to the Canadian border.

Maine's salmon take part in extensive marine migrations, including movements to the waters off western Greenland (Friedland 1994), where they become a small portion of a large mixed-stock complex of salmon from both European and North American sources. Unlike Atlantic salmon populations across the Canadian border from Maine, where 1SW fish are common among spawning adults, about 94% of adults returning to Maine are 2SW fish (USASAC 1999). Thus, the average body size of Maine adults is larger than Canadian adults. Because spawning populations of Maine salmon include several age groups (especially 2SW and 3SW adults but also precocious mature parr[3]), there is considerable exchange of genetic material across age classes (cohorts).

Atlantic salmon spawn in freshwater streams on both sides of the Atlantic (Figure 2). The oceanic fishery in recent years has been concentrated to the west of Greenland, where fish from North America and Europe are encountered (Reddin and Friedland 1999). Natural movement (straying) of spawning

[2] Many Atlantic salmon have escaped from farms off the west coast of North America and concern has been expressed about their becoming established there (e.g., Volpe et al. 2001). Although adult Atlantic salmon have been observed in streams there, they are not wild and have not become established.

[3] Parr are young salmon growing in fresh water. Precociously mature parr are young males that spawn without first migrating to the sea.

fish from Europe to North America, or the reverse, is extremely limited (Reddin et al.1984).

Reproduction

Spawning occurs in autumn, and the eggs develop in gravel nests that are dug by the female. Because Maine's females are large 2SW fish, they deposit about 7,200 eggs. The fry emerge in mid-May and grow into parr. Vertical bars on the parr camouflage them, providing protection from predators. The territorial parr attempt to occupy portions of the river that allow rapid growth on insect fauna. Most parr remain for 2 years in freshwater, before becoming smolts and migrating to the ocean. Occasionally, parr will mature in the stream[5] and can have some success in fertilizing eggs. Survival from the egg to the smolt stage is estimated to be 1.25% (Bley and Moring 1988, Baum 1997), and thus a rough calculation from Baum's data suggests that an average of 90 smolts are produced by a wild Maine 2SW female.

CONSEQUENCES OF SALMON LIFE HISTORY

The salmon life-history pattern is of enormous import for the evolution, survival, and current problems of Atlantic salmon. Because the fish migrate upstream to spawn, they are visible and a source of food and recreation to many people, even those who do not live by the sea. Because salmon spend time in both ocean and freshwater environments, they are subjected to the vagaries of two systems that are only loosely connected. This pattern leads to a kind of double jeopardy and complicates understanding of the factors that affect their populations.

The pattern of homing to their natal streams leads to a variety of local adaptations, including the timing of spawning runs, growth rates, and other life-history features (e.g., Gharrett and Smoker 1993, NRC 1996, and Smoker et al. 1998 for Pacific salmon species; Kendall 1935, Saunders 1981, Heggberget et al. 1986, Verspoor et al. 1991, Kincaid et al. 1994, Webb and McLay 1996, Hutchings and Jones 1998, and Nielsen 1998 for Atlantic salmon; and Allendorf and Ryman 1987 and Taylor 1991 for both Atlantic and Pacific salmon). The occurrence of at least some straying allows the development of what geneticists call a metapopulation structure. The importance of the metapopulation structure for the survival of Pacific salmon species was discussed in an earlier NRC report (NRC 1996), and the conclusions apply to Atlantic

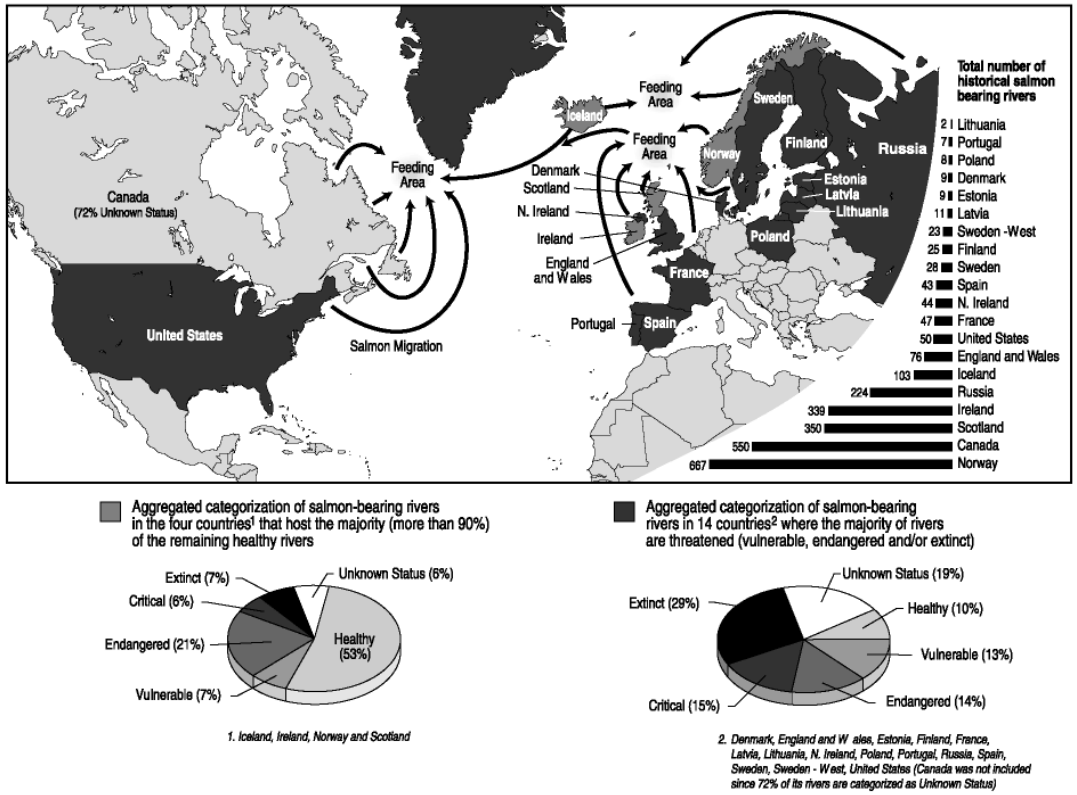


FIGURE 2 Map showing the wild Atlantic salmon's range in 2000 and its known migration routes. Source: *The Status of Wild Atlantic Salmon: A River by River Assessment.* Reprinted with permission from World Wildlife Fund - U.S., Copyright 2001.

salmon as well. Although strays probably have lower reproductive success than fish that are returning to their native streams, they do provide some potential for new genetic combinations—important for the salmon's evolutionary potential in the face of changing environments—and perhaps more importantly, they allow for recolonization of streams if a local population disappears. In some ways, a metapopulation structure can be likened to the structure of a large tree. A few branches can be lost without serious damage, but if only a few branches survive with little communication among them, the tree's survival is in doubt.

The complex physiological transition to salt water at the smolt stage requires suites of behavioral adaptations for navigation and avoidance of predators, including seals, cormorants, and striped bass, and for finding marine foods, including invertebrates and fish. Survival of smolt to 2SW stage would have to be about 2% (based on Baum's estimate of 90 smolts produced per female) to maintain a steady population. Decrease in either freshwater or oceanic survival would cause a decline of Maine's wild salmon populations.

The anadromous pattern, with some repeat spawning, means that counting the fish returning to a stream gives information only on a small part of the population. The rest of the population is either in the river as fry, parr, or smolts or at sea preparing to return. In addition, salmon have overlapping rather than discrete generations. The presence of early maturing males (precocious parr) tends to buffer the population somewhat against random variation among the anadromous (adult) male spawners each generation (Martinez et al. 2000, Garcia-Vazquez et al. 2001). Repeat spawning is important because of the increased egg production of older females and their success in the face of natural selection.

Finally, for Atlantic salmon populations to have colonized and survived for extensive periods near the southern limit of the species' range (currently Maine), they probably had to acquire adaptations to the distinct physical and environmental challenges of local waters. Local adaptations, promoted by strong homing and strong selection pressures, are known for salmon populations throughout the world.

3
Current State of Atlantic Salmon in Maine

WILD POPULATIONS

Maine has the last of the wild Atlantic salmon populations of the United States. Historically, 300,000 to 500,000 adults probably entered U.S. rivers each year (Stolte 1981, Beland 1984). An analysis of the distribution of U.S. Atlantic salmon by the Biological Review Team (1999)—using zoogeographic information to construct ecological provinces, including aquatic ecological units (Bailey 1995, Maxwell et al. 1995)—suggests that historic Atlantic salmon populations were divided into at least three distinct groups of populations: one in Long Island Sound, with eight major river populations, including the Connecticut River; one in Central New England, including the Merrimack River; and one in the Gulf of Maine, including the eight current DPS rivers.

The Long Island Sound populations were extirpated by the early 1800s (Meyers 1994), and the central New England populations by the mid-1800s (Stolte 1981, 1994). The remaining Gulf of Maine populations might have produced 100,000 adults per year, but these numbers have not been seen since the late 1800s. Since the late 1960's, the number of returning adults has been only about 5,000 or fewer. Fewer than 1,000 adults returned each year during the beginning of the period and in 1999 and 2000 (Figure 3). The estimated total return for the eight streams comprising the Gulf of Maine DPS was only 75-110 adults in 2000 (John Kocik, National Marine Fisheries Service, presentation to NRC committee, June 12, 2001).

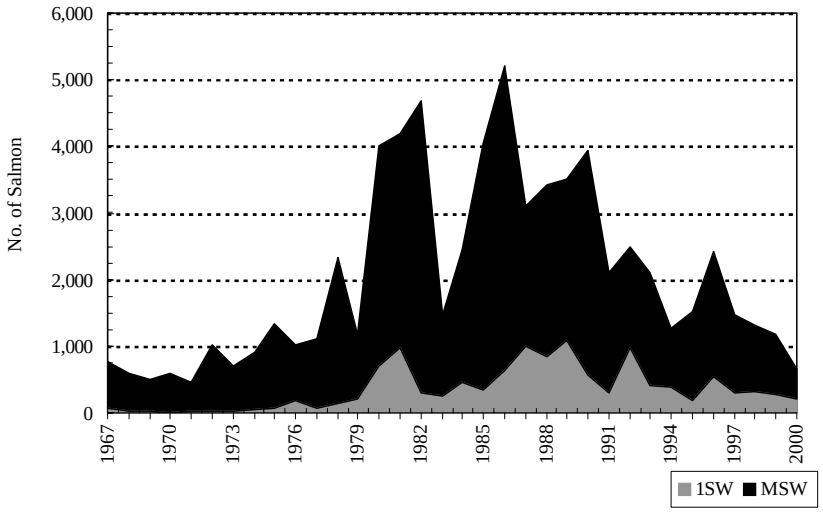


FIGURE 3 Documented adult Atlantic salmon returns to all Maine streams (rod and trap catches combined). These numbers represent minimum adult returns. Source: E. Baum, Atlantic Salmon Unlimited, unpublished material, 2001. Printed with permission of the author.

STOCKING

Augmentation of wild populations with hatchery releases began soon after the first major declines in Atlantic salmon in the 1870s. It is convenient to divide Maine's stocking history into three phases: historical (1871 to 1970); recent (1970 to 1992); and contemporary (1992 to present) (Figure 4). Details can be found in Baum (1997) and the report of the Biological Review Team (1999).

Historical Period (1871-1970)

This period began with the release of parr from Ontario into the Sheepscot River (probably from Lake Ontario[1]). As the import cost was high, a hatchery

[1]The Atlantic salmon in Lake Ontario, extirpated by about 1900, might have consisted of two runs, those that remained in the lake (landlocked) and those that went to the ocean (anadromous). They might have spawned in the same or different

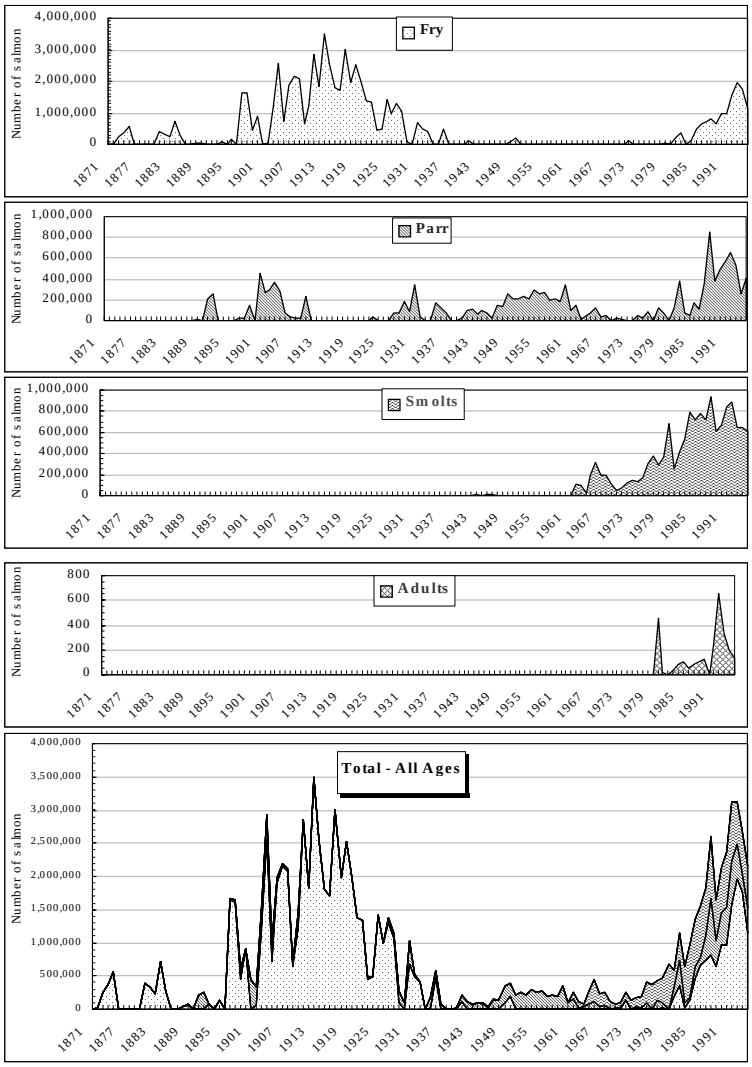


FIGURE 4 Number of Atlantic salmon stocked in Maine rivers, 1871-1995. Source: Adapted from Baum 1997.

tributaries of the lake (Kendall 1935, Parsons 1973, Scott and Crossman 1973). It is not known whether the fish used for stocking purposes in Maine were lake- or ocean-run fish or whether these two possible types were genetically different from each other.

was established at Craig Brook, and the Penobscot River became the primary source of eggs for artificial production for the next 50 years. As runs began to decline in the Penobscot River, during the 1920s and 1930s, Canadian populations (primarily from the Miramichi and Gaspé rivers) became the main sources of eggs for the Craig Brook Hatchery. By the 1940s, Canadian eggs were largely replaced by eggs from the Machias and Penobscot rivers and, to a lesser extent, from the Dennys River. In the 1950s and 1960s, declines in Penobscot runs once again resulted in the use of Canadian Miramichi salmon, supplemented with eggs from the Machias and Narraguagus brood stocks. Throughout this period, hatcheries stocked only fry and parr, and the results were poor. By the late 1960s, stocking had switched largely to smolts, which was followed by the recovery of the salmon run in the Penobscot. The last of the Miramichi-origin salmon were released in Maine in 1968, when the Penobscot population became the main supplier, but Maine salmon could have been infiltrated with Canadian genetic material by then.

Recent Period (1970 to 1992)

This period was typified by an emphasis on producing and releasing smolts, assisted by the Green Lake National Fish Hatchery, and a focus on using Maine sources, primarily the Penobscot but also the Narraguagus and Machias rivers. There was growing recognition that wide geographic movements of genetic material were less than ideal, often resulting in poor returns and creating problems for local adaptation (Ricker 1972, Hindar et al. 1991, Waples and Do 1994, NRC 1996, Utter 1998). By the mid-1970s, the Penobscot brood stock supported essentially all the artificial propagation needs of Maine. During this recent period, Atlantic salmon throughout Maine were managed as a single population for stock-release purposes.

Contemporary Period (1992 to present)

Since 1991, stocking has been river-specific and based on a conservation-hatchery program. The Craig Brook National Fish Hatchery was converted from a single-stock smolt-production facility to a multiple-stock fry-production facility (BRT 1999). Brood-stock collections began with returning adults in 1991, but insufficient numbers led to parr collections in 1992 (Buckley 1999).

The current protocol involves raising (river-caught) parr to adulthood, mating them according to approved protocols of the Maine Technical Advisory Committee (Beland et al. 1997, Copeland et al. 1998), rearing the embryos, and releasing the fry (usually before they begin feeding). Fry stocking began in 1992, and stocking levels had reached target density levels by 1997. There is no indication of a successful increase in returning adults as yet. The shift to a river-specific management and propagation system was based on the premise that naturally spawning fish from even more local waters would provide the best hope for success.

Consequences of Stocking Protocols

Two points about the stocking protocol are important. First, despite 130 years of stocking, using a variety of life stages (fry, parr, smolts, and even adults) and releasing about 120 million Atlantic salmon, the systematic decline in run sizes has not been reversed. That raises the question of whether hatchery stocking has ever had a substantial impact on populations of Atlantic salmon in Maine. Second, the consequences of extensive stocking on the genetic makeup of Maine's salmon are unknown. Has the release into Maine's rivers of large amounts of genetic material, some from outside the region and always with the potential for genetic change through hatchery selection, overwhelmed the gene pool of the aboriginal Gulf of Maine populations?

The Biological Review Team (1999) and Baum (1997) concluded that the hatchery fish have not displaced the local gene pool because of the poor success of historical hatchery stocking and the likelihood that Canadian fish were poorly adapted to Maine streams. In addition, there is now considerable evidence that stocked fish do very poorly (Lieder et al. 1990, Fleming and Gross 1993, Skaala et al. 1993, Fleming et al. 1997, Petersson and Järvi 1997). A review of 31 studies of incursion of hatchery genetic material into wild populations (Fleming and Petersson 2001) reported that 14 studies showed little or no evidence of incursion, despite prolonged hatchery releases. Many of those studies involved anadromous populations. In contrast, 16 of the 17 studies showing an incursion involved nonanadromous populations, suggesting that anadromous populations are more resistant to introgression (see also Utter 2000). If that is true, genetic infiltration of Maine salmon by hatchery releases would be minor. In addition, from 1970 to the present, an average of 84% of stocked Atlantic salmon were of natural origin in the eight Maine DPS rivers

(USASAC 1999). The Gulf of Maine populations might have maintained their genetic integrity largely through natural processes. Although hatchery introductions must have had some impacts, they may have been small.

AQUACULTURE AND ESCAPEES

The second-most dramatic change involving Atlantic salmon in Maine (the collapse of wild populations being the first) is the production of farm-raised Atlantic salmon through aquaculture. Although Maine produced only about 16,400 metric tons (t) of farm salmon in 2000, compared with a global production of more than 800,000 t (Working Group on North Atlantic Salmon 2001), its production was zero as recently as 1986. Five freshwater hatcheries across the state provide smolts for about 600 net-pens floating in sheltered areas, where the fish grow on food pellets broadcast into the pens and reach market size in about 18 months.

If production grows in Maine, additional farm sites in the protected waters along Maine's coast will be needed. The current sites apparently do not have room for additional production. Production is currently concentrated in Washington and Hancock counties—referred to as Downeast Maine—an area that includes five of the eight DPS rivers. Aquaculture in the area could potentially be affected by ESA provisions.

The genetic issue for wild runs is that salmon escape from aquaculture and enter the rivers to spawn (Hansen et al. 1991, Hindar et al. 1991, Carr et al. 1997, Youngson et al. 1997, Gross 1998). These farmed fish are of a different genetic makeup, because they include nonnative strains, because of directed selection by the breeders for traits valuable to the industry (e.g., growth rate, fat content, disease resistance, and delayed maturity), and because of the inadvertent selection by the novel environment (e.g., reduced fright response, disease resistance, and altered aggressive behaviors) (Gross 1998, Fleming and Einum 1997, Johnsson et al. 2001). Those same traits might not be adaptive in the wild.

It is difficult to know what genetic lineages are being used in Maine aquaculture. The Biological Review Team (1999) concluded that there were three brood-stock lines: Penobscot (Maine), Saint John (Canada), and Landcatch (Scotland). The latter is composed primarily of Norwegian strains. The predominant strain was developed from populations from 41 rivers and locations, and was supported in development by the Norwegian government (Gjedrem et al. 1991). Imported sperm of Norwegian origin, via Iceland, also

has been used in recent years. Baum (1998) suggested that European genetic material permeates approximately 30-50% of farm salmon in Maine. The genetic blend can be expected to evolve as time passes.

Farm salmon escape from containment at all life stages, from embryos through adults, despite efforts to design and maintain escape-proof containers. No accurate data are available on escapement in Maine, but data on the number of individuals entering rivers as adults and some data on hatchery escapes are available. Gross (2002) estimated 3% escapement from similar facilities in British Columbia and elsewhere (British Columbia Environmental Assessment Office 1997). Based on the number of fish being raised in Maine waters, 3% escapement in Maine would translate into about 180,000 escapees per year from net pens. Continued improvements are being made that will reduce, but not eliminate, the number of escapees. An escape rate as low as 0.17%, which would be impressive, would still provide 10,000 escapees per year, 100 times the number of adults that returned to spawn in Maine's eight DPS streams in 2000.

These escapees might not have much impact on healthy wild populations, because farm (pen-raised) salmon have shown competitive inferiority in the wild (Fleming et al. 1996, 2000). However, because of the low numbers of wild adults returning to spawn in recent years, farm salmon represent a large proportion (100% in some years) of the adults entering the rivers to spawn. The effect might be ameliorated to some extent by the precocious wild parr in the streams and by the low reproductive success of farm adults (Fleming et al. 1996). In experimental facilities, farm males had only 1-3% of the success of wild males, and farm females had only 20-40% of the success of wild females, with most matings involving wild males (Fleming et al. 1996). In addition, Fleming et al. (2000) showed that farm salmon introduced experimentally into a wild population had only 16% of the success of wild salmon in producing recruits. Thus, it is possible for wild populations to "resist" genetic infiltration by farm fish, but that potential drops as the number of wild fish becomes small, relative to the number of farm fish. Even a 10:1 adaptive advantage for wild salmon might not be sufficient to overcome a 100:1 numerical advantage for aquaculture escapees. It remains unclear to what degree farm salmon have infiltrated wild populations genetically, or conversely, how resistant wild salmon have been to genetic infiltration. Based on samples taken in 1994-1998, genetic infiltration of farm fish into wild Maine populations was minimal (King et al. 1999). However, if salmon farming in Maine expanded further, the numerical impact (among likely spawners) of aqua-culture escapees would have the potential to become significant.

4
Genetics of Wild Maine Salmon Populations

INTRODUCTION

We now turn to a review and analysis of the genetic evidence. The committee's charge is to describe the genetic makeup of wild populations of Atlantic salmon in Maine with a focus on their distinctiveness. In other words, the committee is asked to assess the extent to which Maine populations of Atlantic salmon diverge genetically from other Atlantic salmon populations and among themselves. The question of distinctiveness applies at several levels. First, are North American salmon genetically distinct from European salmon? Second, are U.S. Atlantic salmon (i.e., Maine salmon) distinct from other North American (Canadian) salmon? Finally, to what degree are salmon populations in the eight DPS rivers in Maine distinct from each other? The answers to these questions are relevant to questions about the most effective methods for protecting and restoring wild populations of Atlantic salmon in Maine (e.g., Hedrick 2001), but we defer those issues until the committee's final report. Atlantic salmon currently spawn in North American streams, ranging from Labrador south to Maine. Quebec has a local population in eastern Hudson Bay, and Newfoundland has its own populations, which might have been established by salmon from Europe as well as North America. West Greenland has at least one natural population and Iceland, has several, which are normally considered to be European populations. For purposes of this report, the major question concerns the relationship between Maine populations and neighboring populations in Canada (New Brunswick and Nova Scotia), because of the

extended history of stocking Maine hatcheries with fish from Canadian streams.

During the early European colonization of North America, Atlantic salmon were found as far south as the Connecticut and Hudson rivers, but continuous attempts to reintroduce the species to the Connecticut have failed to establish self-sustaining wild populations, and the southern limit of wild Atlantic salmon is now the Sheepscot River in Maine (Figure 1). The "at least eight [Maine] rivers" listed by NMFS and FWS (DOI and DOC 2000) as containing wild salmon populations are (from west to east) Sheepscot, Ducktrap, Penobscot, Narraguagus, Pleasant, Machias, East Machias, and Dennys. The Saint John River, whose mouth is in New Brunswick, drains areas in Maine's northeastern highlands, but its salmon are considered to be among New Brunswick's populations. The Penobscot and its tributaries harbor the largest populations in Maine, and those from the Narraguagus, Machias, and Dennys are substantial. Those from the other watersheds are smaller (Maine 2000). Occasionally, Atlantic salmon have been seen in the Androscoggin, Kennebec, Union, and several smaller rivers, but they probably include strays or aquaculture escapees. Those additional rivers might well figure in recovery plans, but with the exception of the lower Kennebec and its tributaries, they are not thought to support wild populations.

The Evidence

Much of the evidence on genetic distinctiveness of Atlantic salmon populations is based on laboratory analyses of the variations in gene products (i.e., proteins) taken from samples in the field. In some cases, the genetic material itself (DNA) is analyzed for variation. In either case, the variation observed is compared among populations, and a variety of tests are performed to decide whether the populations are statistically distinct from each other and how accurately an individual salmon can be identified correctly with its source population. Although there might be some relationship between the genetic variation detected in these analyses and adaptively significant differences among the various populations, none can be inferred from these analyses. The differences could be due to random processes (sampling effect or genetic drift), and the markers themselves are thought to be adaptively neutral. In other words, most of the laboratory analyses of genetic variation discussed below cannot provide information on the degree to which different populations have adapted to different local conditions. They can provide information on the degree of isolation of populations—and isolation of populations is a prereq-

uisite for the development of genetically based adaptive differences in them. Analyses can also describe patterns of variation that can be compared with other sets of populations about which more is known.

Stronger inferences about adaptive differences among populations can be obtained. The simple observation that populations differ in phenotypic traits that can affect fitness (such as fecundity, time of spawning, body size or shape, and growth rates) is not sufficient to infer adaptive genetic differences among them, because those traits can also be affected by environmental conditions. However, if salmon from different populations continue to show differences when reared in similar environments ("common-garden" experiments) or if the differences can be shown to segregate genetically in breeding experiments, then the observed differences are more likely to reflect genetic adaptation. This kind of information is not yet available for Atlantic salmon in Maine.

Several earlier genetic data sets provide us with information on the geographic structure of genetic variation in Atlantic salmon, and they are useful in setting the stage for more in-depth (and more recent) analyses using microsatellite[1] DNA markers of the North American populations. A brief overview of those earlier studies is given below.

Allozymes

Allozymes (short for allelic enzymes) are protein gene products and can be used as indicators of genetic variation. In many cases, different forms of genes produce enzyme variants that can be detected in the laboratory. Those variants seldom have known adaptive significance, but like the other markers discussed below, they might be physically linked on the same chromosome to gene variants that are adaptively important, and they do provide information that can be used to infer differences among individuals and populations.

The allozyme work reported to date on Atlantic salmon over their natural range (Ståhl 1987; reviewed in Davidson et al. 1989) and on Downeast Maine salmon populations (May et al. 1994 and references therein) shows that North American and European continental populations are divergent and that populations from the Baltic diverge from those in the eastern North Atlantic (Ståhl

[1]Short repeated sequences of two to four bases (the building blocks of DNA) that provide multiple alleles for each of many genetic loci. They provide ample genetic variation to characterize genetic differences between individuals, both within and among populations. Currently, microsatellite data are the best available for population screening for genetic variation.

1987). Analysis of populations within regional zones, defined along national lines in Europe and along provincial and state lines in North America, indicates that there is substantial genetic subdivision within continental collections. The spatial scale represented by national regions in Europe is greater than that represented by provincial and state counterparts in North America. There is genetic divergence from watershed to watershed, within regions, and where people have looked, even among tributaries within a watershed. There is also divergence among populations that spawn at different times. This typical pattern has been attributed to precise homing of salmon to their natal streams (Ståhl 1987, Allendorf and Waples 1996, Nielsen 1998).

Mitochondrial DNA (mtDNA)

Mitochondria are cellular organelles that contain genetic material (DNA). MtDNA is maternally inherited as a unit. The various markers of the mtDNA genome are free of the recombination that characterizes all the other types of variation under discussion. It conveys strong information about female lineages, but nothing about male lineages. Like allozymes, variants in that DNA material can provide information on population divergence. The mitochondrial genomes of European and North American populations can be effectively discriminated by the presence or absence of a single RFLP[2] marker of mtDNA (Bermingham et al. 1991). North American populations are dominated by two haplotypes[3] and European populations by one, although the European haplotype has been recovered in Newfoundland (King et al. 1999). There are less-categorical genetic markers that we can use to distinguish statistically among regional and subregional populations within continents (Hovey et al. 1989, Palva et al. 1989, McVeigh et al. 1991, King et al. 1993, Pálsson and Árnason 1994, O'Connell et al. 1995, Tessier et al. 1995, Nilsson et al. 2001). The essential pattern of these studies is described by King et al. (2000), who partitioned the

[2]Restriction fragment length polymorphism. Bacteria have enzymes that cut DNA strands containing particular sequences—useful in degrading invasive viral DNA. These (restriction) enzymes can be used to assay DNA samples. Genetic variation in the population ensures that some individuals contain DNA sequences recognized by the enzymes, while some do not. The DNA is cut into fragments wherever the restriction enzyme encounters its specific recognition sequence. The position of the resulting DNA bands on a gel indicates the individual's genotype.

[3]Combinations of genetic markers that are linked closely enough along the mtDNA molecule to be inherited as a single unit.

mtDNA haplotypic variation among continents, regions, and watershed populations with the use of molecular analysis of variance. (Amova was used to partition the molecular genetic variation into components; see Excoffier et al. 1992.) The majority of the variation (68%) is attributable to continental divergence, almost enough to be invariably diagnostic; 8% is attributable to population differences within continents (both regional and interwatershed variation), and 24% is attributable to variation among individuals within single populations. If we examine just the variation within the North American collection, 2% of the variation was attributable to Maine versus Canadian divergence, and about 26% to population divergence within either Maine or Canada. The remaining 72% of the variation was found within single populations.[4] Within Maine populations, only 2% of the variation was attributable to divergence among watersheds, 12% was attributable to variation among tributaries in the same watershed, and 86% was attributable to variation within a tributary. Within European populations, virtually none of the variation was attributable to regional (national) divergence, 19% was attributable to divergence among watersheds within regions, and 81% was attributable to variation within single watersheds.

Other Genetic Markers

A diagnostic genetic distinction between European and North American populations of Atlantic salmon was also established by an early study of ribosomal RNA variation using RFLPs (Cutler et al. 1991). A later study of single-locus minisatellite[5] DNA markers showed virtual separation of European and North American populations but with a low frequency of European alleles in Newfoundland (Taggart et al. 1995). An early analysis (Schill and Walker 1994) using RAPD[6] markers of samples from the Sheepscot, Ducktrap, Penobscot, Narraguagus, Pleasant, Machias, East Machias, Dennys, and Saint John rivers showed that genetic divergence among watershed populations

[4]In salmon, the highest percentage of genetic variation is normally among individuals in a population.

[5]Long sequences of DNA repeated sequentially. Some individuals have more copies of the repeat element than others, leading to DNA fragments that move differently on an electrophoretic gel. Comparisons of minisatellite banding patterns can be used as "genetic fingerprints" and to measure genetic differences.

[6]Randomly Amplified Polymorphic DNA. Small segments of DNA replicated (amplified) to large quantities. As with other similar markers, variation is detected by the presence or absences of bands on a gel.

represented 5.7% to 8.0% of the total variation, depending on which rivers were included in the test. This result is comparable to corresponding allozyme partition of variation. Interestingly, the Saint John River sample was differentiable from the others but not as different from the populations in nearby Maine watersheds as from the population in the more distant Sheepscot River. No European versus North American contrast was tested, the distinction having been firmly established by others as described above.

Recent Microsatellite Analyses

Microsatellite DNA markers, like the other markers described above, are assumed to be adaptively neutral. Recently, the North American populations have been sampled more intensively, and genetic assays have benefited from the higher resolution available from microsatellite markers. King et al. (2001) measured the relative amounts of genetic variation found within single populations, among watersheds, among North American provincial and state populations, and between European and North American collections. Such measurements have been made in various ways, the simplest of which is Amova (Excoffier et al. 1992). The results are summarized in Table 1. The results are highly significant,[7] with $p < 0.0001$. European and North American populations are divergent enough (22% of the variation) to make it virtually impossible to mistake a North American genotype for a European genotype or vice versa. As mentioned above, European and North American fish can be artificially hybridized, but hybridization would not occur naturally because of the vast separation of spawning habitats. If it were to happen in North American

[7]The variation percentages extracted from an Amova table are the average per-locus figures. It is quite usual (for any single locus) to have the majority of polymorphic variation within populations. In comparing populations, however, it is important to remember that these are multi-gene breeding collections. The among-population divergence in allele frequencies, when multiplied over many loci, translates into "gene pools" that are substantially (or sometimes) completely non-overlapping in their genetic constitution (Smouse et al. 1982; Smouse and Chevillon 1998). The allele-frequency differences between European and North American populations are large enough that a salmon sampled from North America would almost never have a multi-gene combination that would occur in any European population, and vice versa. The differences between Canadian and Maine populations are smaller, and those among populations in Maine streams (e.g., Sheepscot and Narraguagus) are still smaller. The degree of gene-pool non-overlap increases with the size of the inter-population percentage of the variation.

waters because of escape and interbreeding of aquacultural stock, the F_1 (first-generation) hybrids would be fairly obvious genetically, as are artificial F_1s (King et al. 1999).

European salmon are genetically so different from North American salmon that it should be easy to detect their presence (as aquacultural escapees) among wild spawning adults. Even F_1 hybrids between North American and European salmon should be evident. Detecting the presence of farm fish of Penobscot or St. John origin will be more problematic, because the genetic differences from the wild fish will be smaller (King et al. 1999). The presence of advanced generation hybrids will be difficult to identify with certainty. Further genetic characterization of aquacultural stocks used in North America is needed.

From Table 1a, we discover that Φ_{CT} (the fraction of total variation among individuals that is accounted for by continental average differences in allele frequencies) = 0.219, while Φ_{PC} (the fraction of the within-continent variation that separates populations) = 0.07. The fraction of the total variation that is within populations is $1 - \Phi_{PT} = 0.726$. From Table 1b, confined to North American variation, we discover that Φ_{CT} (the fraction of North American variation that is due to mean regional (provincial or state) differences in allele frequencies) = 0.032, while Φ_{PR} (the fraction of the within-region variation that separates populations) = 0.032. The fraction of total North American variation that is within populations/drainages is $1 - \Phi_{PR} = 0.939$. The corresponding values for European variation are Φ_{CT} (the fraction of European variation that is due to mean national differences in allele frequencies) = 0.061, while Φ_{PR} (the fraction of the within-nation variation that separates populations) = 0.060. The fraction of total European variation that is within populations/drainages is $1 - \Phi_{PR} = 0.886$. These Φ-values are analogous to F-statistics (Excoffier et al. 1992), and they are all highly significant, as determined by permutational testing.

Within the North American collection, the differences among provincial and state collections of populations are smaller, representing about 3% of the total variation. Divergence among watersheds within a province or state accounts for another 3% of the variation. That raises the question of whether drawing political lines around collections from different watersheds is the most sensible way to delineate regional collections. Within-watershed variation represents the remaining 94% of the variation. The corresponding numbers for European populations are 6%, 5%, and 89%, respectively, but the European

TABLE 1 Molecular Analysis of Variance (Amova) for Microsatellite Genotypes

Source of Variation	Variance Components	Percentage of Variation	Φ-Statistic Estimates
Continental and Infracontinental Analysis[a] $(1 - \Phi_{PT}) = (1 - \Phi_{PC}) \times (1 - \Phi_{CT})$			
Among continents	1.12	21.88	$\Phi_{CT} = 0.219$
Among populations	0.28	5.49	$\Phi_{PC} = 0.070$
Within populations	3.72	72.63	$\Phi_{PT} = 0.274$
North American Analysis[b] $(1 - \Phi_{PC}) = (1 - \Phi_{PR}) \times (1 - \Phi_{RC})$			
Source of Variation	Variance Components	Percentage of Variation	Φ-Statistic Estimates
Among provinces/states	0.12	3.15	$\Phi_{RC} = 0.032$
Among populations	0.12	2.99	$\Phi_{PR} = 0.032$
Within populations	3.56	93.86	$\Phi_{PC} = 0.061$
European Analysis[c] $(1 - \Phi_{PC}) = (1 - \Phi_{PR}) \times (1 - \Phi_{RC})$			
Variation Source	Variance Components	Percentage of Variation	Φ-Statistic Estimates
Among countries	0.29	6.13	$\Phi_{RC} = 0.061$
Among populations	0.25	5.28	$\Phi_{PR} = 0.060$
Within populations	4.15	88.59	$\Phi_{PC} = 0.114$

[a]Continental and infracontinental component.
[b]North American provincial variation partitioned into components for populations (streams) and variation within populations.
[c]European provincial variation partitioned into components for populations (streams) and variation within populations.
Source: Adapted from King et al. 2001.

(national) regions are physically more separated than North American (provincial and state) regions. The North American and European popu- lations are much more divergent than the regional collections within a continent, and watershed-specific populations within a province or state are less divergent

still. This pattern is fairly typical for salmon and their relatives, including various Pacific salmon species (Ryman 1983, Allendorf and Waples 1996). Today's pattern is typical of anadromous fish species in general and salmon in particular (Ståhl 1987).

It is useful to compare the recent microsatellite results with older allozyme and mtDNA results. The nuclear (allozyme and microsatellite) markers tell a consistent story, again with $p < 0.0001$. The mtDNA markers show more continental divergence and about the same watershed-to-watershed divergence, but they show less variation within watersheds than do the allozyme studies. The RAPD studies indicate that 5.7% to 8.0% of the variation in populations from the Saint John River and southwestward is accounted for by watershed-to-watershed differences, the rest being accounted for within populations.

Assignment Analyses

Another approach to determine the level of genetic divergence among populations, particularly when separation is less than complete, is to determine the likelihood that one fish can be identified correctly as to its population of origin based on the genetic information. The degree of confidence with which one can assign a particular fish is a measure of the nonoverlap of the gene pools (Smouse and Chevillon 1998). Early work of this type used genetic-distance methods (Spielman and Smouse 1976, Smouse et al. 1982), but more recently, likelihood procedures have been used more often (Paetkau et al. 1997).

Regional Groupings

We consider first the regional groupings, nested within North America and European collections. On the basis of the genetic markers, individual fish can be assigned to the regional population to which they are most similar (e.g., Brown et al. 1996, Paetkau et al. 1997, Smouse and Chevillon 1998). If there were no genetic divergence among the 12 collections, one would expect a successful assignment rate of 1/12 (8%, ignoring continental divergence) or 1/6 (17%, comparing only within a continent).

There is no overlap between North American and European populations (Table 2), an observation that is in keeping with the large among-continent

variance components in Table 1.[8] More interesting here is that the observed correct assignment rates within a continent range from 62% to 100%, a telling demonstration that the provincial and state collections (in North America) and the national collections (in Europe) are strongly separable among themselves (Table 2). Although the European national collections (not the focus of this report) are more strongly separated than provincial and state collections in North America, the latter are markedly distinct from each other, despite an extended history of wide movements of Canadian genetic material for restocking purposes in Maine.

Watershed-Specific Populations

Assignments to watershed for large numbers of Maine salmon are provided in Table 3. The degree of correct assignment varies from 10% for the Pleasant to 84% for the Penobscot rivers, but the average correct assignment rate (49% overall) is consistent with expectations based on experience with other populations and is less than the regional assignment rate. Published sample sizes are smaller than ideal here, and it would be useful to extend this sort of interwatershed assignment analysis to the populations of the Canadian provinces. Despite voluminous and homogeneous stocking for most of these watersheds until 1990, substantial subregional divergence remains. This situation indicates either that a previously existing structure has persisted because of intense selection against hatchery fish or that watershed-specific stocking since 1990, coupled with homing, has allowed reemergence of a subregional structure. Whether the current structure is due to adaptation of distinct populations to divergent selective pressures or due to genetic drift, the degree of regional substructure is startling, particularly in view of the stocking and run-size history of the last several decades. Interestingly, a study of DNA from old scale samples of an endangered Danish Atlantic salmon population (Nielsen et al. 1997) showed that ancestral genotypes can persist despite years' of extensive stocking. Similarly, Guffey (2000) reported that brook trout (*Salvelinus fontinalis*) populations in the southern Appalachians were able to retain much of their ancestral southern genotypes despite 35 years of

[8]Newfoundland salmon are grouped with North American salmon, despite some probable contribution to their gene pools of European origin. They would not be confused with European salmon.

TABLE 2 Numbers of Genetic Assignments of Individual Atlantic Salmon to Regional Populations

	ME	**NB**	**NS**	**QB**	**NF**	**LB**	**IC**	**NO**	**FN**	**SC**	**IR**	**SP**
ME	**571**	36	9	1	12	5	0	0	0	0	0	0
NB	8	**76**	7	23	5	3	0	0	0	0	0	0
NS	5	8	**88**	6	2	0	0	0	0	0	0	0
QB	0	25	5	**89**	1	2	0	0	0	0	0	0
NF	5	7	5	4	**71**	**1**	0	0	0	0	0	0
LB	0	6	1	2	2	**32**	0	0	0	0	0	0
IC	0	0	0	0	0	0	**95**	1	0	0	0	0
NO	0	0	0	0	0	0	0	**99**	0	2	0	0
FN	0	0	0	0	0	0	0	0	**61**	0	0	0
SC	0	0	0	0	0	0	0	0	0	**38**	8	7
IR	0	0	0	0	0	0	1	0	0	11	**48**	4
SO	0	0	0	0	0	0	0	7	0	5	4	**68**
Percentages Correctly Assigned												
	89	**62**	**80**	**73**	**76**	**71**	**99**	**98**	**100**	**72**	**75**	**81**

Abbreviations: ME, Maine; NB, New Brunswick; NS, Nova Scotia; QB, Quebec; NF, Newfoundland; LB, Labrador; IC, Iceland; NO, Norway; FN, Finland; SC, Scotland; IR, Ireland; SP, Spain.

Source: Adapted from King et al. 2001.

heavy stocking of northern genotypes. These results lend credence to the Atlantic salmon results in Maine.

This same assignment can be taken to examine temporal and subwatershed spatial variation in genetic frequencies. If temporal and spatial variations are large enough within a watershed, then the divergence among watersheds becomes less relevant. The results in Table 3 might be due only to uncertainties associated with the small samples within different watersheds. The available data on subwatershed divergence are sparse, but there is a recent study of temporal variation within and among tributaries (subwatersheds) of the Penobscot River (Spidle et al. 2001), and the data are instructive, as far as they go. The basic idea is to treat each collection separately, assign individuals to collections (based on genotype), and then ask where the misclassified individuals should be placed. If substantial numbers of the misclassified individuals are placed in the wrong tributary, that argues against the reality of genetic differences among them and in favor of sampling effects. If they are placed in the wrong cohort but the correct tributary, that argues in favor of the sort of cohort-to-cohort temporal variation expected of an organism with overlapping generations. The results are shown in Table 4.

Table 4 shows that a substructure exists even within the Penobscot River,[9] bothamong tributaries and among cohorts; genetic differences among populations in different tributaries are a reality. On the other hand, the misclassifications are placed preferentially into other cohorts in the same tributary. That is exactly what one would expect, given the homing behavior of salmon. The yearly cohort samples from a given tributary vary somewhat, but they show more in common with other cohorts from the same tributary than they do with salmon from other tributaries. This striking result has been seen in other anadromous species (Brown et al. 1996), and it argues strongly for thegenetic cohesion of these populations, notwithstanding predictable variation among cohorts (Figure 4) (Jorde and Ryman 1995, Ryman 1997). Evidently, the temporal differences within a tributary are smaller than the average differences among tributaries. The correct assignment rates in Tables 2, 3 and 4 are

[9]Note that for the Penobscot that we have 141 = (22 + 119) assigned to the correct cohort, and 63 = (23 + 40) to the incorrect cohort, an indication of partial separation, but also an indication of considerable overlap. The departure from the 102:102 null expectation is significant, but the gene pools represented by cohorts of the same population are not as different as tributary to tributary differences. The same is true of the Kenduskeag, where the assignment numbers are 41 = (16 + 25) correct and 26 = (12 + 14) incorrect. The Cove Brook cohort collections are more strikingly different.

TABLE 3 Numbers of Genetic Assignments of Individual Atlantic Salmon to Watershed-specific Populations

	KB	**SH**	**DT**	**PB**	**NA**	**PL**	**MA**	**EM**	**DE**
KB	**140**	1	5	8	2	6	5	14	4
SH	7	**9**	0	2	0	4	2	6	0
DT	4	1	**17**	0	4	0	1	3	0
PB	13	1	0	**124**	1	3	0	5	0
NA	20	4	3	4	**38**	16	11	19	1
PL	13	2	1	1	2	**30**	3	7	2
MA	7	2	0	2	5	4	**3**	3	0
EM	24	4	1	5	7	6	5	**41**	4
DE	6	0	0	2	0	1	1	3	**17**
	Percentages Correctly Assigned								
Watershed	**74**	**30**	**57**	**84**	**32**	**56**	**10**	**41**	**57**

Abbreviations: KB, Kennebec; SH, Sheepscot; DT, Duck Trap; PB, Penobscot; NA, Narraguagus; PL, Pleasant; MA, Machias; EM, East Machias; DE, Dennys
Source: Adapted from King et al. 1999, Table 18.

collectively much larger than random expectation ($p < 0.01$) by formal chi-square tests.

TABLE 4 Numbers of Genetic Assignments of Individual Atlantic Salmon to Cohort and Tributary of the Penobscot River

	Pb-95	**Pb-96**	**Cv-93**	**Cv-94**	**Cv-95**	**Cv-98**	**Kd-96**	**Kd-97**
Pb-95	**22**	*23*	0	0	1	0	0	3
Pb-96	*40*	**119**	0	0	1	0	1	2
Cv-93	0	0	**10**	*2*	*1*	*0*	0	0
Cv-94	0	0	*3*	**24**	*3*	*0*	0	0
Cv-95	0	1	*0*	*3*	**14**	*0*	0	0
Cv-98	0	1	*0*	*2*	*1*	**34**	0	0
Kd-96	0	3	0	0	0	0	**16**	*12*
Kd-97	1	0	0	0	0	0	*14*	**25**

Percentages Correctly Assigned

Cohort	**44**	**69**	**67**	**80**	**74**	**90**	**52**	**60**
Tributary	**92**		**95**				**92**	

Note: Numbers assigned to the wrong cohort but within the correct tributary are shown in *italics*.
Abbreviations: Pb, Penobscot mainstem; Cv, Cove Brook; Kd, Kenduskeag Stream.
Source: Adapted from Spidle et al. 2001.

OVERALL SUMMARY OF GENETIC STUDIES

The overall pattern that emerges from all of these studies is that European and North American populations are substantially different. Newfoundland salmon are a partial exception; they appear to be of North American ancestry for the most part, but they show some evidence of European genetic contribution, in keeping with their post-glacial colonization history.

The microsatellite DNA data are the most extensive and therefore our inferences are based most strongly on them. The other data are consistent with the microsatellite DNA data, however, which increases our confidence. For allozyme and microsatellite markers, there is broad regional divergence among European (national) populations and somewhat less among North American (provincial or state) populations, the latter having less spatial separation. The RAPD results parallel those for the allozymes and microsatellites to the extent that the sampling frame was comparable. For mtDNA markers, the patterns within Europe and within North America are reversed with respect to the allozyme and microsatellite results, more variation occurring among North American regions than among European regions. The mtDNA markers show about the same watershed-to-watershed divergence as the nuclear markers, but they show less variation within watersheds. Within either continent, genetic similarities are slightly higher in populations from different tributaries within major watersheds than in populations from different major watersheds.

Collectively, the results show the same pattern of hierarchical genetic divergence as that shown by the previous extensive allozyme data (May et al. 1994 and references therein) and that shown by the less-extensive protein and DNA analyses of Atlantic salmon from North America (e.g., Verspoor 1986, Ståhl 1987, Bermingham et al. 1991, King et al. 1993, Schill and Walker 1994, Kornfield 1994, Taggart et al. 1995, McConnell et al. 1997, King et al. 2000). The results show large divergence between continental (North American and European) populations; broad regional divergence on both sides of the Atlantic, European national populations being about twice as divergent as North American provincial and state populations; and comparable interwatershed divergence within Maine and within the Canadian provinces. In addition, intertributary divergence in a major watershed (e.g., the Penobscot River) is sometimes substantial, with predictable temporal variation within a given sampling locality. This is the typical pattern seen in salmon and their relatives (Ryman 1983, Ståhl 1987, Allendorf and Waples 1996).

5
Quality of the Data

BACKGROUND

Reservations have been voiced about the studies on Maine Atlantic salmon reported to date. Some relate to the sampling regime in the rivers, some to the reality of population delineation, some to the laboratory assays being used, and others to the statistical analyses. Some issues are more serious than others, but all deserve comment. First, however, we discuss the importance for these studies of taking into account the consequences of the overlapping generations of salmon, in particular the temporal variability that is introduced.

OVERLAPPING VERSUS DISCREET GENERATIONS

Although the genetic processes in populations with overlapping and nonoverlapping generations are identical in many respects, the temporal allele-frequency dynamics are different. Without proper consideration of such differences, it can be difficult to interpret population data (Ryman 1997). When considering temporal changes in populations, textbooks on population genetics refer almost exclusively to organisms with discrete (nonoverlapping) generations. However, unlike organisms with nonoverlapping generations, those with overlapping generations have a demographic age structure with the following features: (1) the total population is made up of individuals that belong to different age classes; (2) a restricted set of age classes participates in reproduction, and their relative contributions to the newborns of the next year vary; (3) as

a consequence, the parents of a particular year-class (cohort) do not represent a random sample from the entire population of the previous year; and (4) as a further consequence, allele- frequency differences among the newborns of consecutive years do not necessarily indicate temporal frequency changes for the total population (Jorde and Ryman 1995).

In an age-structured population, the collection of individuals for genetic analysis frequently focuses on a restricted set of age classes. For anadromous Atlantic salmon, commercial catches at sea will not include the youngest age classes, whereas electrofishing on the nursery grounds in streams will primarily sample subadult cohorts. Comparisons of different collections (say commercial catches and nursery-ground collections) are not comparisons of comparable age classes. Allele-frequency dynamics within a generation are such that comparisons among cohorts must be made with care.

To illustrate the different genetic dynamics of populations with discrete versus overlapping generations, Ryman (1997) designed a model of typical Atlantic salmon populations and simulated the effects over 200 years (Figure 5a). The most important observation from Figure 5a is that a population with overlapping generations displays considerably larger allele-frequency shifts from year to year than does a population with discrete generations of the same effective size. Moreover, there is a tendency for temporal correlation in the overlapping generation population; allele frequencies tend to fluctuate in a cyclical fashion that is not expected when generations are discrete.

Clearly, population size is not the only determinant of the amount of temporal allele- frequency change when generations overlap, because the total population does not represent a single genetically homogeneous unit. Rather, it consists of several age classes (six in the Ryman 1997 simulation; Figure 5b) that are produced, partly or completely, from different sets of parents, and, therefore, the different cohorts might exhibit different allele frequencies. The amount of temporal noise in the allele frequencies of the total population thus depends on the age structure (the relative proportions of the different age classes). Likewise, periodicity is introduced, because each cohort of progeny (young fish) is similar not to the cohort of progeny in the previous year but instead to the cohort to which most of the *breeding fish* of the previous year belonged. Those breeders were young fish several years previously. The magnitude of the variation and the length of the periodic cycle are functions of the number of cohorts present among the breeders (in any given year) and their relative contributions to reproduction. The amount of temporal allele-frequency noise also depends on the age-specific birth and death rates of the particular population (Jorde and Ryman 1995).

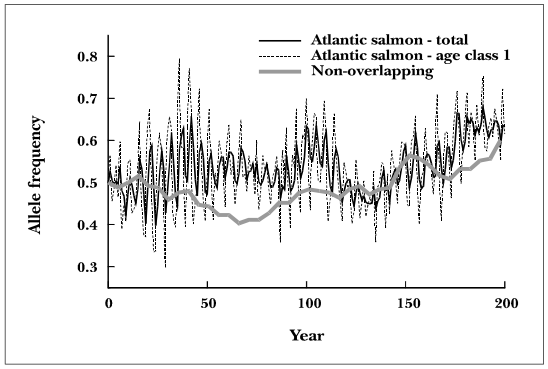
For a population with overlapping generations, allele-frequency homogeneity among age classes is not expected for a neutral locus. The random component of temporal variation is increased—especially in small populations—by genetic variation among cohorts (Waples 1989, Jorde and Ryman 1995, Ryman 1997). Conversely, the observation of statistically significant differences should not automatically be viewed as evidence of gene flow, natural selection, nonrandom sampling (say of family groups), or an alarmingly small effective population size (Waples 1989, Ryman 1997). Several reports have been published comparing multiple samples from the same population for allele-frequency heterogeneity for genetic loci (Ryman 1997, and references therein). Too often, rejecting the null hypothesis of allele-frequency homogeneity (statistically significant heterogeneity) has led investigators to the conclusion that something is "wrong" with the population or with the samples. Explanations have ranged from very small effective population sizes (genetic drift) to natural selection on the loci examined, to straying among populations, and to familial sampling. Such conclusions might not be warranted, given the temporal heterogeneity expected with overlapping generations.

FAMILIAL STRUCTURING OF SAMPLED FISH

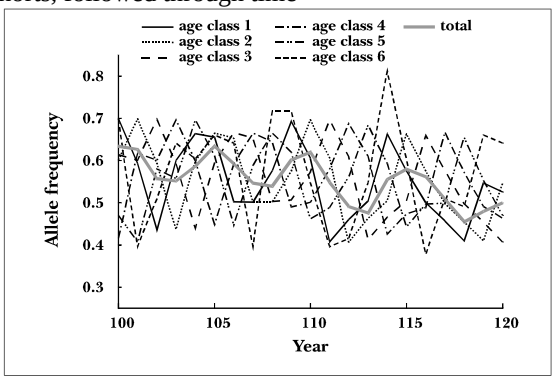
All routine statistical tests assume that any collection of fish gathered as a single population sample (a location and a year, a sampling event) represents a genetically random draw. Basically, the assumption is that the collection of individuals in the sample is as likely to be a genetic draw of individuals from that year and location as any other draw from that year and location. The sample is considered to be exchangeable with other samples. All the fish within a population will eventually become related to some degree or another, but that is not the issue. The question is whether an exchangeable sample, relative to the blend of genotypes available, is drawn. If the fish sampled in one event are more closely related to each other (family members) than an exchangeable draw, then subsequent analysis will yield a higher estimate of the genetic divergence among samples, because the genetic divergence shown by closely related fish from a single sample will be less than that shown by unrelated fish from different samples (Jorde and Ryman 1995, Hansen et al. 1997).

Concern has been expressed that some of the samples collected in the past might not have met the exchangeability criterion (Kornfield 2000, Gold 2000).

(a) Total population



(b) Single cohorts, followed through time



(c) First and second age classes for each year

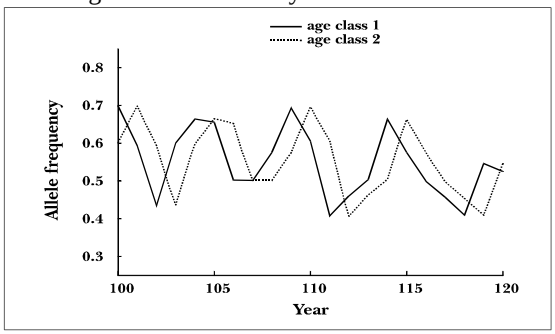


FIGURE 5 Simulated temporal allele-frequency shifts of a selectively neutral allele in populations of identical effective size (N_e = 124) with overlapping and discrete generations. The initial allele frequency is p = ½: (a) total population results (and those for age class 1 of the model with overlapping generations), each population simulated or 200 years; (b) contrast of single-cohort frequencies, followed through time, for years 100-120; (c) contrast of age classes 1 and 2 for years 100-120. Source: Ryman 1997. Reprinted with permission from ICES *Journal of Marine Science*; copyright 1997, Academic Press.

Females typically lay several nests within a single redd,[1] along a short linear segment of stream (#3 meters). Hatching fry from a single redd, representing a blend of full- and half-siblings, tend to school in the vicinity of that redd. Parr are territorial and tend to spread out a bit more from their hatching locality. Still, within a small stretch of stream, they may be related to some degree. Smolts are solitary and are more likely to spread out over longer stretches of the stream. Adults, collected either at the spawning sites or downstream at the weirs, can be viewed as representative, relative to the array of genotypes drawn by homing to a particular natal tributary.

Many of the samples studied in previous work, involving fry, parr, smolts, and adults, have been collected by electrofishing, accomplished by walking along the stream with an electric wand that is effective over a small area (1-2 square meters). The total area of coverage can be large, but if fish within the treated area have a higher probability of being close relatives than a random draw of fish from the total population, any interpopulation measure of genetic divergence will be increased. Thus, fry collected by electrofishing might yield overestimates of the degree of population differentiation. Collections of parr are probably less affected, and collections of smolts and adults are probably not affected to any significant degree. Given the sorts of mixed cohort sampling that have been routine in the past, the distinctiveness measures among populations might be slightly increased, but the observed degree of divergence among sampled populations appears to be too large for familial sampling to be a major cause of its magnitude. We view the results as (possibly) minimally inflated but as nevertheless credible and compelling. We suggest that fry and parr should be collected from as wide a spatial area as possible within each local population as a means of avoiding undue sampling of any one family.

POPULATION DESIGNATIONS

The results to date for Atlantic salmon in Maine make clear that there is genetic divergence among tributaries within large watersheds. This divergence is a common result for fishes of the salmon family. The results for the Penobscot River (Table 4), for example, are clear that the entire watershed does not contain a cohesive, randomly mating population. The key to this situation is that genetic organization of salmon populations is hierarchical and orga-

[1]A group of egg pits, or nests, prepared by a single female. The eggs in each nest are fertilized by one or more males—not necessarily the same male for each nest.

nized by watershed. Straying occurs, but it is most likely from one spawning segment of stream to the next, a bit less from one tributary to the next, and even less from one watershed to the next. One could imagine situations where a large watershed might best be treated in tributary-specific fashion, as in Pacific salmon species (e.g., Waples and Smouse 1990). That might make some sense within the Penobscot and within the Machias-East Machias complex, but some degree of lumping within a watershed seems unavoidable, particularly if brood stock are to be reared en masse at the hatchery.

It also is obvious that watersheds within a region are divergent from those of the next region (on average), but what does regional divergence mean, when the regions are defined by political (rather than biophysical) criteria? For example, the Saint John River includes two political units but is assigned to the regional (political) unit that contains the mouth of the river. An intriguing question is whether the current (politically delimited) regions make optimal biological sense and whether the management of Maine, New Brunswick, and Nova Scotia salmon might be coordinated profitably. Toward that end, it would be productive to extend the assignment analyses of Maine fish to include the relevant watersheds in New Brunswick and Nova Scotia. Having said that, it is clear that the collective Maine population is distinct from the collective New Brunswick and Nova Scotia populations.

LIMITATIONS OF GENETIC ASSAYS

The laboratory aspects of genetic assays have received some criticism as well (Kornfield 2000, Gold 2000). All the genetic assays in current use have limitations, most associated with scoring the genetic markers on electrophoretic gels. This technique separates variant DNA fragments or proteins on a gel when they migrate at different rates in response to an electric charge. The basic operating premise of such work is that if two individuals each have a band (a scorable element) at the same position on the gel, the two bands represent the same thing. For allozyme protein markers, different alleles (slightly different amino acid sequences) can move the same distance on the gel under the influence of an electric charge. Allozymes are dealt with as classes, each of which is defined by its (joint) position on an electrophoretic gel. Although the formal population genetic analysis is not sensitive to the hidden variation within bands (i.e., the variation that is not separated by electrophoresis), there is a natural frustration with the inability to see everything.

The availability of DNA markers has greatly improved the resolution of

genetic variation, and most laboratories have now switched to DNA markers, because the allozyme classes can be separated into the sequences associated with different protein alleles. To screen a population involving thousands of fish, the required DNA sequencing would be too expensive. What has been done instead—with a variety of molecular-genetic techniques, such as RFLPs and RAPDs, with minisatellites, and (most important for this report) with microsatellites—is to revert to gel-scoring techniques for DNA fragments, this time separated by size (number of nucleotides in the fragment). The lumping (different DNA sequences of similar size) is less than it is for allozymes (different protein sequences of the same charge), but the principle that equal movement implies genetic identity is the same, even though the principle implies some loss of resolution. As mentioned earlier, the population genetic theory is impervious to the nuances of lumping. Some variation is missed but what is seen is clearly present. Microsatellite methods reveal substantially more genetic variation than earlier allozyme methods. Interestingly, however, the conclusions drawn from the two sorts of data are qualitatively similar.

Kornfield (2000) rightly called attention to a related issue with the early microsatellite results of King et al. (1999). With microsatellites, adjacent bands are very close together on the gel. There are so many alleles (fragments with different lengths) for some of the loci that it can be difficult to make small distinctions on the gel, and one is forced to "bin"[2] alleles, placing alleles of almost the same size into allelic classes and reducing the number of alternatives under consideration. Even though there is variation within the allelic classes, it is necessary to construct such classes. This is again the issue of lumping. Although it is tolerable, it is exacerbated, because alleles between two adjacent bins (sizes) can be misallocated.

A variety of related scoring problems can beset such work, particularly with large-scale population-screening efforts. Microsatellite markers provide a rich source of genetic information and permit population assessments that were previously impossible, but the best laboratory control available must be used. Fortunately, quality control continues to improve, and the obstacles are all surmountable with some extra attention given to detail in the laboratory. The more recent published reports (King et al. 2001, Spidle et al. 2001) have profited from Kornfield's (2000) timely reminders.

[2]The binning approach to the analysis of genetic information has been widely used in forensics (e.g., NRC 1992).

STATISTICAL CONSIDERATIONS

Previous work has involved many statistical tests. A trio of statistical issues have been raised about such tests, all of which must be dealt with in future work (Kornfield 2000, Gold 2000). First, some sample sizes have been small, particularly in view of the number of genetic characters examined (Smouse and Chevillon 1998), calling into question the precision and power of the resulting statistical tests. In some cases, small sample sizes are unavoidable, because population sizes are themselves small, but whenever possible, efforts should be made to obtain sample sizes of at least 100 fish per collection.[3]

Second, the analysis of rare alleles or haplotypes is particularly sensitive to sample-size effects. Given a collection of S alleles or haplotypes from the population being sampled, the expected number recovered in a sample of size N is computable (Chakraborty et al. 1988), but because the number of fish sampled from a single population is sometimes smaller than the number of potential alleles or haplotypes, no more than a few of the latter can be seen. In general, the common (high frequency) types are seen consistently with a smattering of the rare types. Although the pattern of rare types is intriguing, too much has been made of that information in view of available sample sizes; any inference attributed to the presence or absence of rare types in small samples is unreliable. The genetic resolution for measuring population divergence is in the high-frequency genetic markers, and the results in Tables 1-4 are insensitive to rare alleles or haplotypes.

The third criticism of much of the previous work is that too many hypotheses were being tested. The nominal probability levels are too generous, and it would seem that more conservative Bonferroni or even stepwise Bonferroni procedures (Rice 1989) would be better. Moreover, with K population samples, there are $K(K-1)/2$ pairwise tests of divergence but only $(K-1)$ independent contrasts, and the full set of pairwise tests is highly intercorrelated. For multipopulation comparisons, a standardized test criterion (Smouse and Williams 1982) might be better. For broad survey work, a small number of independent tests, such as those available from F-statistics, Amova, or assignment procedures, would be best. The available population genetic data depart so far

[3] There are many more fry in the rivers that can be sampled than returning adults, so this is a reasonable expectation.

from the null hypothesis (of homogeneity) that substantial divergence is obvious by inspection.

USEFUL INFERENCE

A statistically significant difference is not necessarily biologically meaningful. With large-enough sample sizes, any difference could be statistically significant. The results of the studies reviewed here are highly statistically significant. The question is whether the differences are large enough to be biologically useful. The assignment tests in Tables 3-5 show that the multiple-locus gene pools of the various populations are substantially nonoverlapping. The differences are large and strongly suggest biologically important genetic isolation among the populations.

6
Conclusions

The genetic studies reviewed above lead to consistent conclusions: there is large divergence between continental populations of Atlantic salmon in North America and Europe, considerable divergence among regional populations in Canada and in Maine, and divergence between populations in different watersheds in Maine. In addition, divergence can be substantial among populations from different tributaries within a watershed, and temporal variation occurs within a given sampling locality. Thus, the committee concludes that wild populations of Atlantic salmon in Maine are distinct from other wild Atlantic salmon populations and that differentiation occurs among populations within Maine.

The question arises whether divergence of the adaptively neutral genetic markers that we describe above indicates anything useful about adaptation (of other loci). This question has serious restoration and management implications. Behind the ESA-important question of "Are they distinct?" are the deeper biological question of "How much does it matter?" and the derivative management question of "How can we best use what we know for restoration?" Here we address only the first question—that is, whether the genetic divergence that exists has any biological importance.

While there is obviously a limit to the adaptive importance we can impute from the population divergence that has been demonstrated, we are reluctant to dismiss local adaptation casually. The pattern of variation is so typical of wild salmon that it suggests considerable genetic cohesion and resilience of the resident populations, in spite of large scale stock releases (over decades) that could only have homogenized the various populations, had they been effective.

Stocking clearly has not been completely effective, as shown by declining run sizes over the last 30 years. Whether today's genetic differences represent a remnant of salmon population structure that predates human intervention, following thousands of years of natural selection and genetic drift, typical of salmon occupying different habitats with a variety of environmental circumstances, or whether they represent five to six generations of genetic drift, exacerbated by an increasingly serious population collapse over a short period, is a question that we cannot answer by genetic characterization of neutral genetic markers alone. Any conclusions we draw about the selection/drift dichotomy will necessarily be circumstantial. Suffice it to say that the patterns of variation we see are typical of wild salmon exhibiting the effects of both selection and drift.

Maine Governor Angus King, in his presentation to the committee on June 12, 2001, asked whether we are dealing with Maine salmon or merely salmon in Maine. The distinctiveness of Maine salmon is important, but it is not the whole question, which we consider to have two parts. For the first part, the genetic evidence available for review indicates that wild salmon swimming in Maine's DPS-designated rivers are genetically distinct from salmon swimming in Europe, from those in Canada, and from those used in the Maine aquaculture industry. Collectively, the data are persuasive on these points, from which we conclude that the natural salmon spawning in Maine's DPS-designated rivers are "Maine salmon," not just "salmon in Maine."

The second part of the question is whether these Maine salmon are mainly hatchery-created mixtures or the results of natural processes—including migration, colonization, natural selection, and genetic drift—that occur in network-connected watersheds. More specifically, the issue concerns the relative importance of natural selection over long periods, which influenced the differentiation of Maine's original salmon populations, versus recent genetic drift or a sampling effect related to small populations. Hatchery supplementation—including the movement and mixing of multiple stocks, adaptation to hatchery husbandry practice, and genetic drift—has the potential to alter the gene pool of wild populations. If salmon in Maine are merely the local "farm crop," rather than largely the result of natural processes, they might not be "Maine salmon." We do not have data to answer this question completely.

We can, however, draw some inference from comparisons of the watershed-specific samples of the current DPS rivers. If Maine salmon are an artificial construct of non-river-specific hatchery supplementation, then the separate watershed-specific populations should be genetically indistinguishable. The genetic evidence available for review indicates that the natural populations

are distinguishable from each other. Moreover, their degree of distinctiveness is typical of that found throughout the remaining world distribution of wild Atlantic salmon. The data suggest that current Maine salmon in the DPS rivers are not mainly hatchery mixtures but rather show the typical metapopulation structure that characterizes wild populations of salmon and their relatives in places where stocking has been absent or insignficant. Maine has wild salmon populations in the eight DPS rivers that are as divergent from Canadian populations and from each other as expected among wild salmon populations elsewhere in the Northern Hemisphere.

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Appendix A

Statement of Task

A multidisciplinary committee will review the available scientific information on the status of Atlantic salmon populations in Maine and, where relevant, in adjacent areas. The committee will assess causes of the declines of their populations and the current threats to the continued survival of salmon, will evaluate the evidence on population structure of those salmon, and will evaluate options for improving the survival of salmon. In assessing information, the committee will identify significant knowledge gaps and suggest additional research that would be important to the conservation and recovery of salmon populations.

Factors to be evaluated include the nature and distinctness of salmon populations in Maine rivers and surrounding areas; the interactions between aquaculture, hatchery, and wild populations; terrestrial and marine environmental factors affecting salmon populations; the effects of changes in the hydrology of Maine's streams on salmon; and the effects on salmon of subsistence, recreational, and commercial fishing in freshwater and ocean areas in and around Maine.

A brief interim report will be produced within 9 months after formation of the committee. The interim report will address the genetic makeup of wild salmon populations in Maine and its possible relationship to recovery activities. A final report at the end of the study will describe and synthesize the information available on the biology of Atlantic salmon, the causes of their population declines, and threats to their continued survival. It will evaluate and describe options for enhancing their continued survival and recovery, and will provide some approximate estimates of the relative costs of the various options.

Appendix B

Meetings and Presenters

First Meeting: June 12-13, 2001, Bangor, Maine

The following individuals made presentations to the committee at the public session that took place on June 12-13, 2001, at the Four Points Sheraton Hotel, Bangor, Maine:

Edward Baum, Atlantic Salmon Unlimited
Elizabeth Butler, Pierce Atwood
Mary Colligan, National Marine Fisheries Service
John Gold, Texas A & M University
Governor Angus King, State of Maine
John Kocik, National Marine Fisheries Service
Irv Kornfeld, University of Maine
Chris Mantzaris, National Marine Fisheries Service
Paul Nickerson, Fish and Wildlife Service
Lee Perry, Commissioner of the Department of Inland Fisheries and Wildlife
Alan Spear, Bangor Hydro-Electric Company
Adrian Spidle, U.S. Geological Survey
Joan Trial, Maine Atlantic Salmon Commission
Fred Whoriskey, Jr., Atlantic Salmon Federation

Second Meeting: September 20-21, 2001

The following sites were visited during a field trip held on September 20, 2001. The individuals who made presentations at the various sites are listed below.

Maine Atlantic Salmon, LLC Facilities, Cross Island, Maine
Alf-Helge Aarskog, Maine Atlantic Salmon, Inc.
Sebastian Belle, Maine Aquaculture Association
Elizabeth Butler, Pierce Atwood
Daniel MacPhee, Maritime Veterinary Services
Stephen Page, Maine Atlantic Salmon, Inc.
Steve Swartz, Maine Atlantic Salmon, Inc.

Small Grower - Blueberry Farm, Whitneyville, Maine
David Bell, Wild Blueberries Commission
Lincoln Sennett, Wild Blueberry Grower
David Yarborough, University of Maine

Cherry Field Foods, Columbia Barrens, Maine
Brad Caswell, Cherryfield Foods
Sid Reynolds, Cherryfield Foods

Pleasant River Wier, Pleasant River, Maine
Joan Trial, Maine Atlantic Salmon Commission

Craig Brook National Fish Hatchery, East Orland, Maine
Tom King, Craig Brook Hatchery
Jerry Marancik, U.S. Fish & Wildlife Service
Ralph Pisapia, U.S. Fish &Wildlife Service

The following individuals made presentations to the committee at the public session, held on September 21, 2001, at the Four Points Sheraton Hotel, Bangor, Maine:

Edward Baum, Atlantic Salmon Unlimited
Kevin Friedland, National Marine Fisheries Service, University of Massachusetts

Andy Goode, Atlantic Salmon Federation
Terry Haines, U.S. Geological Survey
Fred Kircheis, Maine Atlantic Salmon Commission
George LaPointe, Maine Atlantic Salmon Commission
Jeff Reardon, Trout Unlimited