Maine Rivers

Two Reports on Alewives in the St. Croix River:

St. Croix River Alewife – Smallmouth Bass Interaction Study

T.V. Willis

Genetic Analyses of Freshwater and Anadromous Alewife (*Alosa pseudoharengus*) Populations from the St. Croix River, Maine/New Brunswick

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Acknowledgments

The scientific findings of the studies in this report will provide managers, politicians and fishing interests with essential scientific information upon which to begin making cooperative decisions regarding the future management of St. Croix alewives and smallmouth bass.

Maine Rivers gratefully acknowledges the collaborative efforts over the last two years of many individuals and organizations in making possible the scientific studies contained in this report. These studies absolutely would not have been possible without the generous support of the funders listed on the front cover. At the risk of overlooking someone (if so, please accept the apology and gratitude of the editor), Maine Rivers wishes to thank individually the members of the Scientific Advisory Committee, Rod Bradford, Peter Cronin, Eric Hutchins, Rick Jordan, Sandra Lary, Pam Seymour, Lee Sochasky, Tom Squiers and Gail Wippelhauser; others who helped at various important points along the way, Curtis Bohlen, Lynn Dwyer, Ken Elowe, Stewart Fefer, Steve Gephard, Jon Kachmar, Tom Kelsch, Rick Lawrence, Bill Townsend, Joan Trial and Fred Whoriskey; researchers P. Bentzen, I.G. Paterson and T.V. Willis; the guides, conservationists and residents of the St. Croix watershed who provided their knowledge and expertise; and - in memory - Clem Fay for his ever-present insight. Finally, thank you to Naomi Schalit and Lee Sochasky for their vision and dedication in bringing together all the individuals and organizations necessary to develop this essential scientific work.

November 2006

St. Croix River Alewife – Smallmouth Bass Interaction Study
Final Report

Dr. T.V. Willis

FINAL REPORT

to

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St. Croix River Alewife – Smallmouth Bass Interaction Study

Executive Summary

BACKGROUND

This project was implemented to provide critical information needed to bridge a longstanding international impasse in the management of smallmouth bass (*Micropterus dolomieu*) and alewives (*Alosa pseudoharengus*) in the St. Croix River system of Maine (USA) and New Brunswick (Canada)[1]. This controversy of more than two decades duration has involved players ranging from local anglers to high-level federal officials on both sides of the border. It has considerable policy and resource implications to this boundary region and elsewhere in the State of Maine.

In 1981, fishway improvements led to a resurgence in the St. Croix's anadromous (searun) alewife population (see Timeline figure below). A coincident decline in the smallmouth bass population in Spednic Lake was blamed by some on alewives. Public concern over the negative impact alewives might have on the economically critical smallmouth bass sport fishing industry included the perception that the presence of alewives anywhere in the drainage was a risk to the fishery. To address this concern, in 1995 the Maine Legislature passed legislation to block migrating alewives from ascending state-controlled fishways on the St. Croix to reach their spawning grounds. Restricted access to spawning grounds is accepted to be the primary cause of a precipitous decline in the St. Croix alewife population from hundreds of thousands of fish in the mid-1990s to just 900 fish in 2002.

The question asked in all subparts of this project was whether anadromous alewives negatively affect smallmouth bass populations in lakes where they co-occur. Specific questions included:

1. Does the presence of anadromous alewives result in lower condition, length or growth of smallmouth bass?

2a. Does the presence of adult anadromous alewives result in young-of-year smallmouth bass mortality as a result of adult alewife predation?

2b. Does the presence of young-of-year anadromous alewives result in diet overlap between smallmouth bass and anadromous alewives, a component of competition which potentially leads to lower growth or survival?

3. Does the presence of anadromous alewives result in smallmouth bass tournament results that are lower than tournament results in lakes without anadromous alewives?

4. Are landlocked alewives in the St. Croix drainage the result of a shift from an anadromous (seasonal migrant) to a landlocked (permanent resident) life style, or were they introduced from distant landlocked populations?

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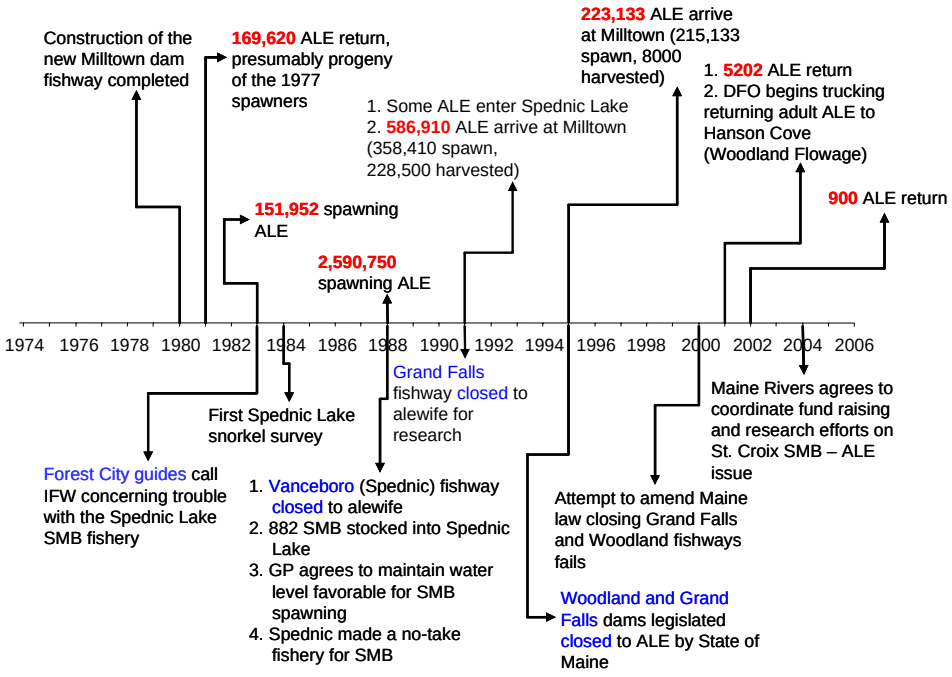
[1] The following organizations were represented on the project's Scientific Advisory Committee: US National Marine Fisheries Service, US Fish & Wildlife Service, Maine Dept. of Inland Fisheries & Wildlife, Maine Dept. of Marine Resources, Canada Dept. of Fisheries & Oceans, New Brunswick Dept. of Natural Resources, and St. Croix International Waterway Commission. The committee's role included substantial contributions to the proposal and work plan used in defining and delivering this project, along with review and commentary of interim, draft and final reports. Data were contributed by state and provincial agencies where indicated. The study was funded, in part, by the Maine Outdoor Heritage Fund, the Gulf of Maine Council on the Marine Environment/NOAA Habitat Restoration Partnership, the National Fish and Wildlife Foundation, the International Joint Commission and the New Brunswick Wildlife Trust Fund. This work is solely the property of Maine Rivers and does not summarize or represent the official or sanctioned position of any other organization.

Timeline of events and management decisions for St. Croix River alewife



Timeline of events in the management of alewives and alewife returns on the St. Croix River. Spawner escapement in any year consists mostly of fish spawned 4-5 years previous (St. Croix International Waterway Commission 2000). ALE = anadromous (searun) alewife, IFW = Maine Dept. of Inland Fisheries and Wildlife, SMB = smallmouth bass, GP = Georgia-Pacific Corporation, DFO = Dept. of Fisheries and Oceans Canada

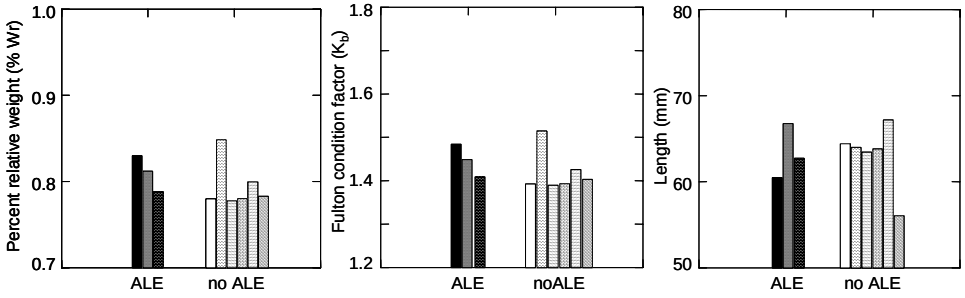
METHODS AND FINDINGS

1. EFFECT OF ANADROMOUS ALEWIVES ON CONDITION, LENGTH AND GROWTH OF SMALLMOUTH BASS BASED ON MAINE DEPARTMENT OF INLAND FISHERIES AND WILDLIFE DATA

Ten lakes were used to study the historic effects of alewives on smallmouth bass populations. All ten lakes were located in Maine and within Maine Department of Inland Fisheries and Wildlife (Maine IFW) resource management Region C. Smallmouth bass and alewives co-occurred in the years covered by the study in three of the lakes, six lakes contained bass but no alewives, and one, Woodland Flowage, always contained bass but had variable alewife populations, including some years with and some years without alewives.

Length and Condition

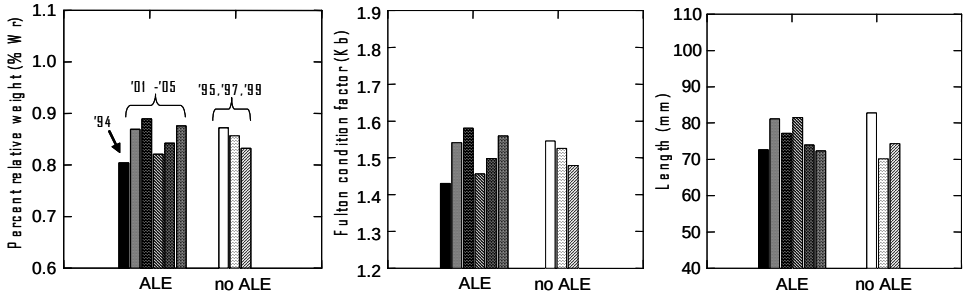
Two measures that estimate the length-weight relationship or "plumpness" of fish for comparison between populations were examined to determine whether young-of-year (YOY) smallmouth bass were in systematically better or worse condition in lakes with alewives as compared to lakes without alewives.



Bass condition and length in lakes with and without anadromous alewives. Individual bars represent lake specific averages of annual data within either grouping. Note the variability between lakes within groups; group themselves had similar averages and distributions. See main text to identify lakes and years of data included.

There was no systematic difference in YOY smallmouth bass length or condition based on the presence or absence of anadromous alewives, nor was there an interaction between lake or year and alewife presence. (An "interaction" is a statistical measure that might point to a more complicated relationship in which alewives might affect bass in different ways in different years or different lakes). Variation within alewife groups between lakes and among years within lakes far exceeded any systematic difference between alewife and no-alewife groups. High inter-lake variability is noteworthy here because much of the approach to alewife management in the St. Croix is based upon the assumption that observations from Spednic Lake in the 1980s are widely applicable to other lakes with co-occurring alewife and smallmouth bass populations.

Since alewife populations varied in Woodland Flowage over the period we examined, it is possible also to look at whether the presence or abundance of alewives affected bass size or condition in that lake.



Smallmouth bass condition and length in Woodland Flowage in years with and without anadromous alewives. Individual bars represent annual averages of data within either grouping. Note that the group had similar averages and distributions. See the main text for the abundance of ALE in Woodland Flowage for the years included.

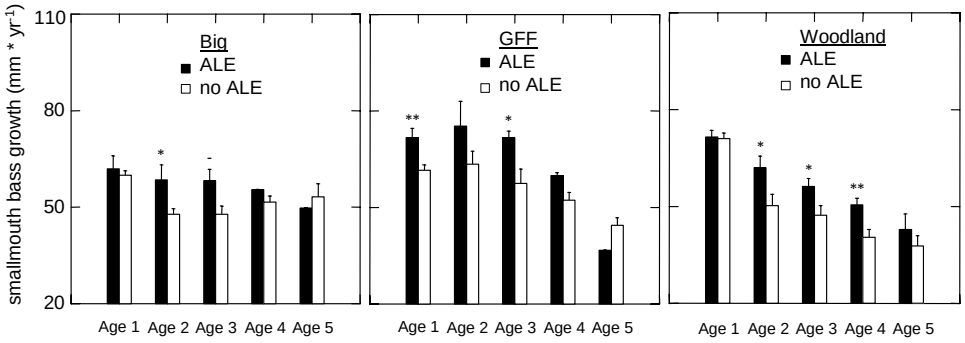
No systematic difference in bass length or condition exists between years with and years without anadromous alewives. Substantial year-to-year variation in the condition of smallmouth bass exists both among years with alewives present and among years in which they were absent. This may be due in part to order of magnitude fluctuations in alewife abundance between the years investigated, i.e., there may have been far fewer alewives in Woodland Flowage between 2001 and 2005 than there were prior to 1995. However, both the years in which bass showed the

best condition and the year in which bass showed the poorest condition were years in which alewives were present. The lowest values of smallmouth bass condition occurred in 1994 when alewives were extremely abundant and in 2003 when the run was extremely depressed.

Growth

Smallmouth bass growth was examined by looking at annual rings on scales sampled from adult bass. Fish scales show annual rings much like the annual rings laid down by temperate zone trees. For the first five years in the life of a bass, the size of each annual ring is related to the overall size of the fish. Thus one can look at a scale collected from an adult fish and derive an estimate of the size of that fish over the first few years of its life. Knowing the size of a fish from year to year makes it possible to calculate how much a fish grew from year to year. Thus from a four year old bass from which a scale sample was collected in the year 2000, one can calculate how much that fish grew as a one year old in 1997, as a two year old in 1998, and so on.

In three of the10 lakes for which historic data were available, alewives were present in some years and absent in others. In those lakes, therefore, one can compare the growth of one year old fish in years in which alewives were present with the growth of one year old fish in years in which alewives were not present, and similarly for older fish.



Growth of age 1 and older smallmouth bass in three St. Croix lakes in which alewives were present in some years and absent in others. Asterisks over pairs of bars indicate that statistically significant differences in growth were observed between years with and without alewives for the specific age of bass in the given lake. Big = Big Lake, GFF = Grand Falls Flowage, Woodland = Woodland Flowage.

In each of the three lakes for which this comparison could be made, growth of one year and older smallmouth bass was either statistically indistinguishable or slightly higher during years in which alewives were present compared with years in which they were absent. Growth was significantly higher for smallmouth bass in the presence of alewives than in their absence for at least one age interval in all three lakes.

2. ADULT AND YOUNG-OF-YEAR DIET HABITS

Seven lakes were chosen to conduct the diet analysis portion of the project: Cathance, Meddybemps, Gardner and Woodland are lakes with co-occurring populations of alewives and smallmouth bass; Big, Grand Falls and Pocumcus are lakes with only smallmouth bass. Alewives

have been stocked into Woodland Flowage up to a density of six fish per acre by the Department of Fisheries Oceans Canada since 2001. Alewives have been excluded from Grand Falls Flowage and Big Lake since 1990 and were excluded from Woodland Flowage between 1995 and 2000.

Adult and young-of-year (YOY) alewives were collected from Cathance, Meddybemps, Gardner and Woodland; YOY smallmouth bass were collected from all seven lakes. Diet was characterized by examining gut contents. Prey items were identified to family whenever possible and organisms in a sample were counted and their length measured. Diet data were expressed as a percent of the index of relative importance (%IRI), which combines several measures of the significance of different organisms in fish diets into a single index value. Potential for competition between young-of-year alewives and young-of-year smallmouth bass was assessed by calculating Schoener's Index, which measures degree of similarity in diet, i.e. diet overlap. Fish diet overlap must be greater than or equal to 60% in order to affect the biology of the species in question. Diet overlap greater than 60% indicates that there is potential for competition but more tests are required to demonstrate whether one species is out-competing another for a resource.

Adult Alewife Diets

Gut contents of adult (anadromous) alewives showed that, unlike some other anadromous fish, they consume a range of diet items once in freshwater. Fish prey made up less than 0.15% of the diet by importance (%IRI), i.e., anadromous alewives were not an important predator on other freshwater fish.

Young-of-year alewife and smallmouth bass diets

Zooplankton was by far the most common diet item for both YOY alewives and YOY smallmouth bass in all lakes. Members of the suborder Cladocera (tiny crustaceans sometimes known as "water fleas") frequently occurred in diets of both species. YOY smallmouth bass, however, also ate numerous invertebrates such as mayfly and midge larvae.

Despite some general diet similarities, diet overlap between YOY alewives and YOY smallmouth bass was only biologically significant (>60%) in Meddybemps Lake. The high diet overlap in Meddybemps reflects the abundance of a single family of zooplankton (the Cladoceran family Sididae) in the diets of both alewives and smallmouth bass. Sididae formed more than 90% of the diet of both species. However, smallmouth bass and anadromous alewives have co-existed in Meddybemps Lake for well over a century.

On the whole, alewives, with their smaller mouths, tend to feed on smaller prey items than do smallmouth bass. Nevertheless, diets of both YOY smallmouth bass and alewives varied substantially from lake to lake, presumably reflecting differences in prey availability. This makes it difficult to predict the degree of diet overlap that might occur between bass and alewives in lakes from which alewives are currently excluded. One approach is to examine the diets of YOY smallmouth bass in those lakes, and compare them to the diets of YOY smallmouth bass in lakes where alewives and smallmouth coexist.

Lake	Big	Grand Falls	Pocumcus
Cathance	10.2%	37.5%	38.0%
Gardner	6.3%	61.9%	84.3%
Meddybemps	3.7%	46.1%	86.3%
Woodland	14.3%	26.3%	9.8%

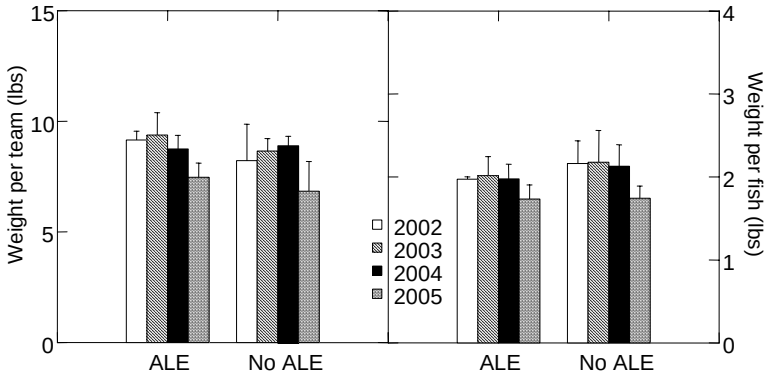
Diet similarity between YOY smallmouth bass in lakes containing alewives (rows) and lakes without alewives (columns), as measured by Schoener's Index. Smallmouth bass in Pocumcus Lake (which lacks alewife) have similar diets to smallmouth bass in Meddybemps Lake, where the two species co-exist, and where diets of YOY alewife and smallmouth bass are similar. However, the diet of smallmouth bass in Pocumcus is also similar to their diet in Gardner Lake, where diet overlap with alewives is less significant.

Since Meddybemps was the only lake sampled in which YOY smallmouth bass diets and alewife diets were ecologically similar, we might be especially interested in looking for any lake in which smallmouth diets are similar to their diet in Meddybemps. Bass in Pocumcus Lake showed 86.3% similarity in diet to bass in Meddybemps. This suggests a chance for significant diet overlap between alewives and bass in Pocumcus. These data must be interpreted with caution, however. First, the smallmouth bass diet in Pocumcus also showed 84.3% similarity with bass diets in Gardner Lake, where bass and alewives did not show ecologically similar diets. Second, smallmouth bass diets may shift in the presence of alewives. Third, where food is abundant and factors other than availability of forage control abundance of YOY smallmouth bass, even significant diet overlap may not signal ecologically important competition.

3. BASS TOURNAMENT RESULTS

Thirteen lakes were used to assess how alewives affected tournament bass fishing. Tournament data were provided by the Maine Blade Runners Bass Club and the New Brunswick Sport Fishing Association. Data consist of entries for individual teams collected at weigh-in, i.e., the total weight of a bag of live smallmouth bass and number of fish in that bag. Ordinarily, entries are restricted to bags containing no more than five fish. This analysis was limited only to tournaments that followed that procedure. Tournament returns, therefore, give an indication of the quality of sport fishing for bass on each lake.

No systematic difference in the weight of tournament entries was observed between lakes with and without alewives. However, tournament returns were lower as a whole in 2005, though not significantly so compared with previous years, regardless of whether alewife were present in the lake or not.

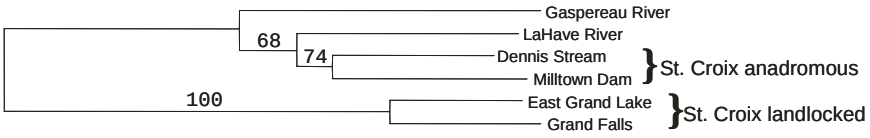


Tournament returns from thirteen lakes in Maine and New Brunswick from 2002 to 2005. Bars represent years; see main text for the list of lakes included in a year of tournament entries.

4. GENETICS OF LANDLOCKED ALEWIVES IN THE ST. CROIX WATERSHED

In a related study, P. Bentzen and I.G. Paterson of Dalhousie University examined the genetics of landlocked and anadromous alewife populations from the St. Croix watershed using 10 microsatellite markers. The purpose of this part of the project was to determine the relationship between landlocked and anadromous alewife populations in the St. Croix. Microsatellites are highly variable stretches of nuclear DNA especially useful for this purpose. The 10 microsatellites were used to characterize alewives from four locations in the St. Croix watershed: anadromous alewives from Dennis Stream and Milltown, and landlocked alewives from East Grand Lake and Grand Falls Flowage. In order to place these four populations into a broader understanding of genetic variation among alewives, two other populations of anadromous alewives from the Gaspereau and La Have Rivers in Nova Scotia were also studied.

Anadromous and landlocked populations of alewives in the St. Croix were found to be genetically distinct. This study concluded that landlocked alewives did not arise from trapped anadromous alewives and instead stem from a separate introduction of landlocked alewives into the watershed. Little, if any, interbreeding occurs between the two life history types, although a lack of data from habitats where the two life history types co-occur in large numbers suggest this conclusion should be seen as preliminary. The populations are sufficiently different from a genetic perspective that it should be possible to identify the life history type of individual St. Croix alewives using genetic data.



Dendrogram showing the relationships of alewife genetic samples. St. Croix anadromous populations are more closely related to anadromous populations from the Gaspereau and LaHave Rivers in Nova Scotia than they are to the landlocked alewives occurring in the St. Croix. Numbers indicate a calculated percent confidence in the dendogram grouping. From Bentzen and Patterson (2005).

Genetic differences observed among alewife populations from different sub-drainages within the St. Croix watershed imply homing of alewives to their natal streams and, consequently, at least partial reproductive isolation between spawning runs, even at the level of tributaries within the St. Croix River. However, the degree of genetic differentiation between the two St. Croix samples was small and should be evaluated further. It is also noteworthy that the landlocked alewives in the St. Croix drainage are non-native invaders whose negative effects are well documented in the Great Lakes scientific literature.

CONCLUSIONS

1) We found no evidence from available historic data for Downeast Maine lakes that the presence of alewives systematically harmed smallmouth bass in terms of length, condition or growth.

2a) Fish constituted only a tiny proportion of the diet of adult anadromous alewives. Alewives were not significant predators on smallmouth bass.

2b) In most lakes, young-of-year smallmouth bass and young-of-year alewives did not have an ecologically significant overlap in diet. In the one lake in which diets were similar, populations of bass and alewives have coexisted for over a century. Based on one year's data, therefore, competition for food between the two species does not appear to be important.

3) Smallmouth bass tournament returns in the past few years have been similar in lakes with and lakes without alewives, suggesting that the quality of sport fishing for bass does not differ systematically between lakes with and lakes without anadromous alewives.

4) Landlocked alewives are genetically distinct from the anadromous alewife populations in the St. Croix and in other investigated watersheds. They are almost certainly the result of an independent introduction of landlocked stock from lakes outside the watershed and not the result of a shift in alewife life history strategy within the watershed.

St. Croix River Alewife – Smallmouth Bass Interaction Study Final Report

INTRODUCTION

This project was implemented to provide critical information needed to bridge a longstanding international impasse in the management of smallmouth bass (*Micropterus dolomieu*) and alewives (*Alosa pseudoharengus*) in the St. Croix River system of Maine (USA) and New Brunswick (Canada)[1]. This controversy of more than two decades duration has involved players ranging from local anglers to high-level federal officials on both sides of the border. It has considerable policy and resource implications to this boundary region and elsewhere in the State of Maine.

In 1981, fishway improvements led to a resurgence in the St. Croix's anadromous (searun) alewife population (See Fig. 1 timeline). A coincident decline in the smallmouth bass population in Spednic Lake was blamed by some on alewives. Public concern over the negative impact alewives might have on the economically critical smallmouth bass sport fishery included the perception that the presence of alewives anywhere in the drainage was a risk to the Spednic Lake fishery as well as to the fisheries in Woodland Flowage, Grand Falls Flowage and Big Lake. To address this concern, in 1995 the Maine Legislature passed legislation to block migrating alewives from ascending state-controlled fishways on the St. Croix to reach their spawning grounds. While this action was not supported by the fisheries agencies on either side of the border nor corroborated by published research, it addressed a perceived management need and public interest expressed by the sport fishing lobby in the area. Restricted access to spawning grounds is accepted to be the primary cause of a precipitous decline in the St. Croix alewife population from hundreds of thousands of fish in the mid-1990s to just 900 fish in 2002 (Fig. 2) .

A five-agency proposal to conduct staged alewife releases into St. Croix waters and study the effects on resident smallmouth bass populations was rejected by the Maine Legislature in 2001. The International Joint Commission and others have investigated alternate means to address this impasse, unsuccessfully. To prevent possible extirpation of the anadromous alewife run and to minimally retain other ecosystem benefits, the Canadian government has, since 2001, trucked a portion of the remaining small alewife run past the first Maine barrier to limited (5.28 km^2 or 2.04 mi^2) spawning habitat in Woodland Flowage. The fish are prevented from moving above this flowage by a barrier at the next upstream dam at Grand Falls that is maintained by the State of Maine.

[1] The following organizations were represented on the project's Scientific Advisory Committee: US National Marine Fisheries Service, US Fish & Wildlife Service, Maine Dept. of Inland Fisheries & Wildlife, Maine Dept. of Marine Resources, Canada Dept. of Fisheries & Oceans, New Brunswick Dept. of Natural Resources, St. Croix International Waterway Commission. The committee's role included substantial contributions to the proposal and work plan used in defining and delivering this project, along with review and commentary of interim, draft and final reports. Data were contributed by state and provincial agencies where indicated. The study was funded, in part, by the Maine Outdoor Heritage Fund, the Gulf of Maine Council on the Marine Environment/NOAA Habitat Restoration Partnership, the National Fish and Wildlife Foundation, the International Joint Commission and the New Brunswick Wildlife Trust Fund. This work is solely the property of Maine Rivers and does not summarize or represent the official or sanctioned position of any other organization.

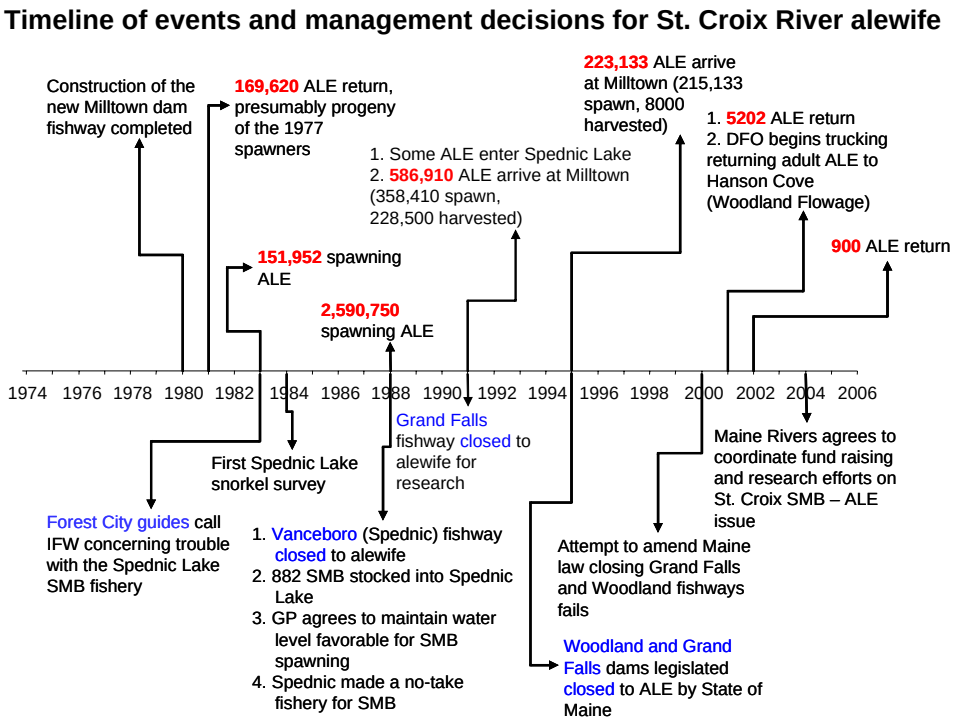


Figure 1: Timeline of events in the management of alewives and alewife returns on the St. Croix River. Spawner escapement in any year consists mostly of fish spawned 4-5 years previous (St. Croix International Waterway Commission 2000). ALE = anadromous (searun) alewife, IFW = Maine Dept. of Inland Fisheries and Wildlife, SMB = smallmouth bass, GP = Georgia-Pacific Corporation, DFO = Dept. of Fisheries and Oceans Canada

Confounding this issue is the recent appearance of landlocked alewives in the upper portion of the St. Croix watershed, beginning in the mid 1990s. This non-native fish has spread to other parts of the system, raising concerns about its added impact on resident fish populations. One concern is whether landlocked alewives are descendents of anadromous alewives trapped in the upper portion of the St. Croix River system. This issue is addressed fully in Bentzen and Paterson (2005); brief summaries of that report appear in the results and discussion sections of this text.

Current Maine policy regarding the interactions between smallmouth bass and alewives in the St. Croix River is based on observations made in Spednic Lake in the early 1980s. The current management regime and many of the attitudes toward alewives are based on the hypothesis that alewives compete with smallmouth bass, the effect of which was a reduction in smallmouth bass production in Spednic Lake. Several year classes of smallmouth bass were absent from that lake in 1984 and 1985 at the start of a Spednic Lake SCUBA Planer Board Survey (Smith 1987). These observations were concurrent with large runs of alewives in the system and observations of large schools of YOY alewives in Spednic Lake. Smallmouth bass and YOY alewives overlap spatially in the lake littoral zone. The concerns of some bass anglers, fishing guides and resource managers in the St. Croix River area were that alewives compete with smallmouth bass, either as adults that may eat YOY smallmouth bass or as YOY that compete with YOY smallmouth bass for food.

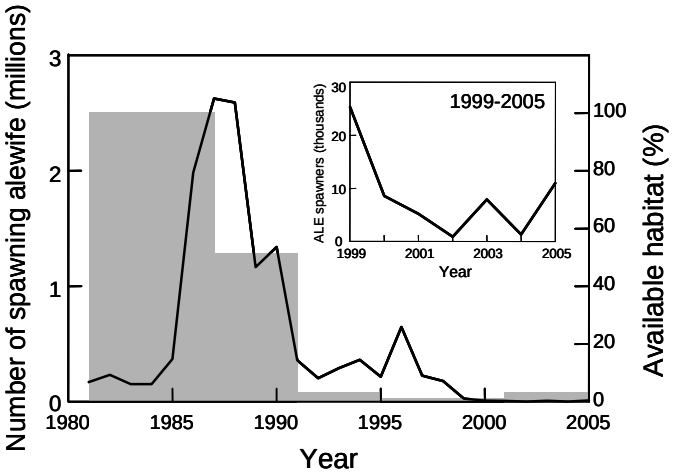


Figure 2: Alewife spawning escapement (left axis, solid line) and available spawning habitat (right axis, shading) graphed against year in the St. Croix River system. The inset shows spawning escapement in number of alewives between 1999 and 2005. Spawning alewives were counted at the Milltown Dam fishway by the St. Croix International Waterway Commission. The maximum spawning area was equal to Woodland Flowage, Grand Falls Flowage, Big Lake and Spednic Lake. The minimum spawning area was equal to waters between Milltown Dam and Woodland Dam.

The goals of this project were: 1) analyze existing agency data for evidence of interactions between anadromous alewives and smallmouth bass, 2) collect and analyze new data concerning the diet habits of adult anadromous alewives, young-of-year anadromous alewives and young-of-year smallmouth bass, 3) analyze existing data from New Brunswick and Maine smallmouth bass tournaments for evidence of interactions between anadromous alewives and smallmouth bass and 4) collect and analyze genetic data to determine whether the St. Croix's landlocked alewife population originates from its anadromous alewife population. The hypothesis tested in all subparts of this project was that anadromous alewives negatively affect smallmouth bass populations in lakes where they co-occur. Specific hypotheses assessed whether:

1. The presence of anadromous alewives resulted in lower condition, length or growth of smallmouth bass,

2a. The presence of adult anadromous alewives resulted in young-of-year smallmouth bass mortality due to adult alewife predation,

2b. The presence of young-of-year anadromous alewives resulted in diet overlap (a component of competition) between smallmouth bass and anadromous alewives,

3. The presence of anadromous alewives result in smallmouth bass tournament results that are lower than tournament results in lakes without anadromous alewives,

4. The presence of landlocked alewives in the St. Croix drainage is indicative of a shift from an anadromous (seasonal migrant) to a landlocked (permanent resident) life style in the upper reaches of the drainage.

METHODS

1. EXISTING AGENCY DATA ANALYSIS

Lakes

Ten lakes were used to establish historic effects of smallmouth bass – alewife interactions on smallmouth bass populations. All lakes were located in the Maine Department of Inland Fisheries and Wildlife (Maine IFW) resource management Region C. Lakes ranged in size from 5 km^2 (2 mi^2) to 13 km^2 (5 mi^2), see Table 1. Six lakes were mesotrophic based on data available in the PEARL online database (http://pearl.maine.edu/) and the rest were oligotrophic. Anywhere from four to twelve years of young-of-year smallmouth bass data and three to eight years of adult smallmouth bass data were available (see Table 1 for lakes and years and Figure 3 for a map of region).

Table 1: Background information on lakes included in the young-of-year smallmouth bass analysis and the age-at-length analysis. Trophic index is based on secchi depth information except for Pocomoonshine Lake where the Chlorophyll-*a* based trophic index is reported. YOY = young-of-year, SMB = smallmouth bass.

Lake	Watershed	Anadromous alewives present	Area[1] (km^2)	Mean depth (m)	Trophic index	Years adult SMB sampled	Years YOY SMB sampled
Beech Hill	Union	No	5.47	13.4	30 oligotrophic		1999-2002
Big	St. Croix	Until 1990	41.7	3.7	59 mesotrophic	1991, 1993, 1995, 1997, 1999, 2001, 2003	1994-2005
Branch	Union	No	10.94	11.9	19 oligotrophic		1995-2002
Cathance	Dennys	Yes	11.76	7.3	10 oligotrophic		1995-2003
Grand Falls	St. Croix	Until 1990	27.08	3.0	62 mesotrophic	1989, 1993, 1995, 1997, 1999, 2001, 2003	1994-2005
Green	Union	No	12.1	13.4	25 oligotrophic		1994-1998, 2000-2003
Meddybemps	Dennys	Yes	27.38	4.3	44 mesotrophic		1992, 1995-2003
Pocomoonshine	East Machias	Yes	9.97	4.3	40 mesotrophic		1992, 1995-2003
West Grand	St. Croix	No	58.03	11.3	mesotrophic		1994-2005
Woodland	St. Croix	Yes (to 1994; 2001 to present), No (1995-2000)	4.75	4.6	--	1990, 1994, 1997, 2000-2004	1994-1995, 1997, 1999, 2001-2005

[1] Square kilometer (km^2) equals 247 acres or 0.4 mi^2. Metric measures are used in this paper, in accordance with international scientific reporting conventions.

Sample collection: Historical Maine IFW data

The objective of Hypothesis 1 was to analyze existing data collected since approximately 1990 for indications that the presence of alewives negatively affected either the condition or growth of smallmouth bass. In particular, this objective addressed the concern that the development of young-of-year smallmouth bass populations was hindered by the presence of alewives. Maine IFW provided smallmouth bass data for condition (YOY) and growth (adult) analysis for ten lakes and three lakes, respectively, in Hancock and Washington Counties in Region C (see Table 1).

Condition data were collected by electrofishing (backpack or boat) up to 50 young-of-year smallmouth bass per year in early fall after growth for the year had ceased or nearly ceased. Fifty fish were not always caught: collection efforts ceased after an appropriate amount of effort had been expended. Collected fish were returned to the lab alive. In the lab fish were sacrificed, measured to the nearest mm and weighed on an electronic balance to the nearest 0.01 grams. Large fish (> 85mm) suspected of being age 1+ were aged by scale and excluded from the data set if found to be older than one year.

Growth data were collected from adult smallmouth bass in the spring before growth commenced for that year. Approximately 100 smallmouth bass were angled from Big Lake, Grand Falls Flowage and Woodland Flowage (see Table 1 for years of available data). One hundred fish were not always caught: collection efforts ceased after an appropriate amount of effort had been expended. Scales were collected from below the right dorsal fin. Before reading, scales were cleaned of dried mucus by gently massaging between two fingers under running water, allowed to dry, and then were mounted between two microscope slides. Scales were aged and distance between annuli and scale radius measured for fish up to age five only, in accordance with Maine IFW protocols.

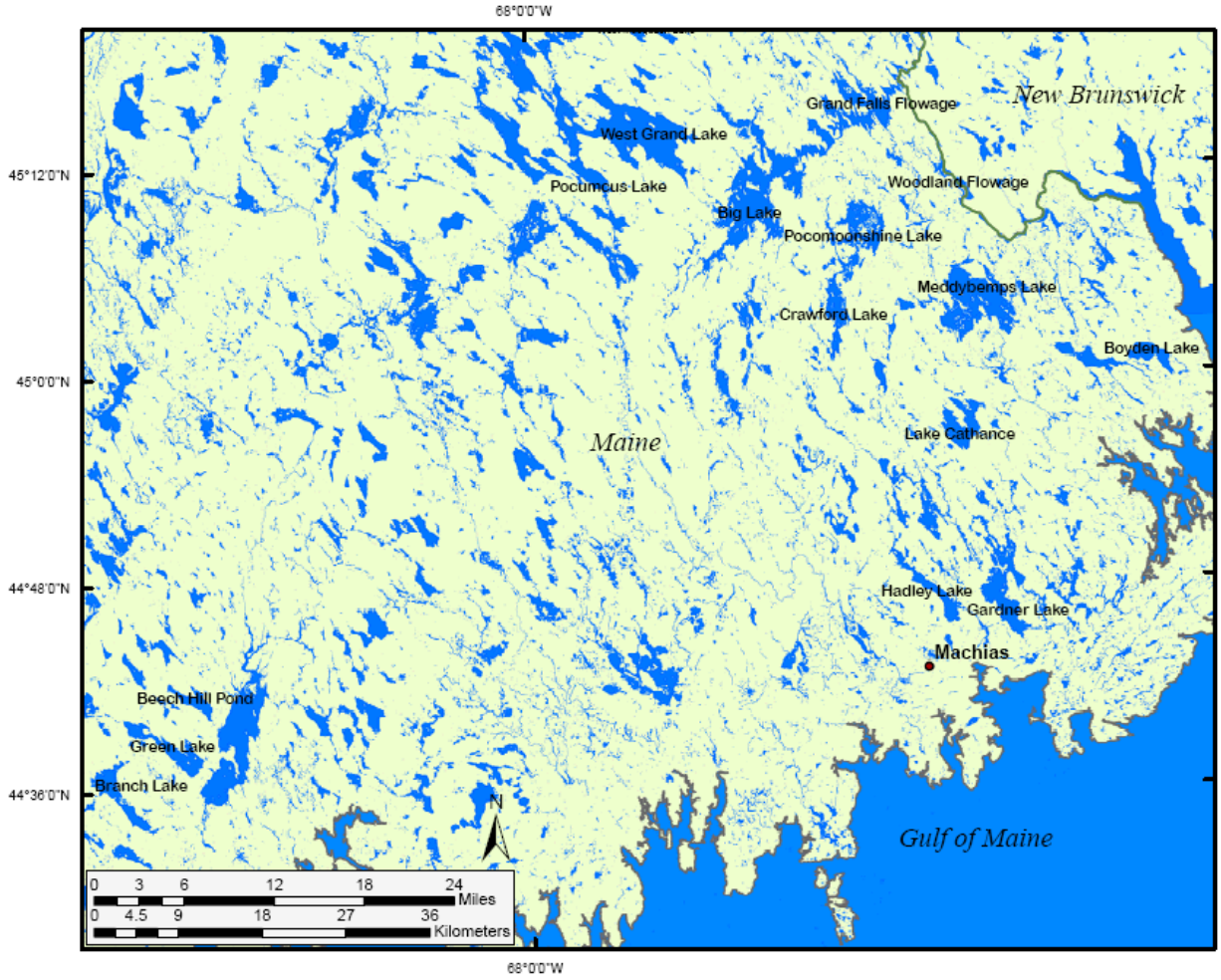


Figure 3: Map of Downeast Maine region with lakes sampled for Maine IFW historical data and lakes sampled for data collected in 2005. For additional lake information and listings of data collected see Tables 1, 2 and 3.

Analysis

Condition

Condition of young-of-year smallmouth bass was expressed as both percent of relative weight (%*Wr*) and Fulton's Condition Factor (K_b). *Wr* is a measure of observed fish weight compared to a standard weight equation calculated for a region. The advantage of calculating *Wr* is that comparing populations against a standard regional length-weight relationship facilitates understanding of among population variation (Murphy et al. 1990, Murphy et al. 1991). The danger of *Wr* is that an inappropriate standard equation calculated from too few populations, poorly documented length and weight data, or populations too far afield may result in inappropriate weight comparisons. To calculate *Wr*, first the observed fish weights in grams (W_{ij}) for fish *j* from lake *i* were regressed against the observed lengths in millimeters ($length_{ij}$) (see Table 1 for years of available data). The regression coefficients m_i and b_i from

(1) $$\log_{10}(W_{ij}) = m_i \times \log_{10}(length_{ij}) + b_i$$

were retained for each lake *i*. Next, 10 mm length intervals were established that matched the longest and shortest fish among all the lakes. Here, the shortest fish collected from any lake was 37 mm and the longest was 106 mm, with the majority falling between 40mm and 90mm; length intervals were established for 30 mm through 110 mm. Regression coefficients m_i and b_i were used to calculate a predicted standard weight for lake *i* using the midpoint of each length interval (the 30 mm interval's midpoint = 35 mm) from the formula

$$(2) \qquad W_{il} = 10^{b_i} \times (length_{il})^{m_i}.$$

W_{il} is the predicted standard weight of length interval *l* in lake *i*, and $length_{il}$ is the standard length of interval *l* in lake *i*. Predicted standard weights for all lakes were grouped by length interval and the upper 75th percentile, or here the three highest standard weights from the 12 lakes, were chosen. Chosen standard weights (n = 24) were regressed against standard lengths and the regression coefficients m_s and b_s were retained from the formula

$$(3) \qquad \log_{10}(W_s) = m_s \times \log_{10}(length_s) + b_s,$$

where W_s are the chosen standard weights and $length_s$ are the chosen standard lengths. Coefficients m_s and b_s were then used to calculate relative weight;

$$(4) \qquad Wr_j = 10^{b_s} \times (length_j)^{m_s},$$

where Wr_j is the relative weight of fish *j*. Percent relative weight was calculated as W_{ij} divided by Wr_j multiplied by 100.

Calculation of Fulton's Condition Factor (K_b) was adapted from Murphy and Willis (1996) using the formula

$$(5) \qquad K_b = \left(\frac{W_j}{L_j{}^m} \right) \times 100{,}000.$$

The term 100,000 is an inverse scalar that makes K_b a more manageable value. K_b is a modification of the Fulton Condition Factor (Anderson and Neumann 1996) in that the scaling coefficient is calculated from the grand population, i.e., all fish from multiple populations collapsed into a single length-weight relationship, rather than m = 3. For this data set m = 2.962.

Statistical analysis consisted of two-way Analysis of Variance (ANOVA) on the average condition of young-of-year smallmouth bass in a lake in a year. Annual averages were computed by lake for length and condition measures that were then grouped into alewife and no alewife categories. Factors in the ANOVA model were lake and alewife presence/ absence. Woodland Flowage was omitted from this analysis because it neither fell into the 'alewife' nor 'no alewife' category, but was treated separately in its own analysis. For Woodland Flowage, differences in length, %Wr, and K_b were explored with alewife escapement and year as factors in a two-way ANOVA. Spawning escapement, i.e. the number of alewives either allowed to pass Woodland Dam or stocked into Woodland Flowage at Hanson Cove by the Department of Fisheries and Oceans Canada, was log(x+1) transformed prior to analysis. K_b and %Wr were arcsine transformed according to the modified procedure in Sokal and Rohlf (1995).

Growth

Smallmouth bass growth was calculated from estimated length-at-age. Length-at-age was calculated using the scale back-calculation formula:

$$(6) \qquad L_{ij} = 21 + \left(\frac{A_{ij}}{r_j} \right) \times \left(L_j - 21 \right)$$

where the L_{ij} is the length-at-annuli (or length-at-age) of fish j at annulus i, A_{ij} is the distance between annuli i and the focus of fish scale j, r_j is the radius length of fish scale j, and L_j is the length at capture of fish j. Growth was calculated by:

$$(7) \qquad G_{ji} = L_{ji+1} - L_{ji}$$

where G_{ij} is the growth of fish j at annuli i and L_{ji} is the estimated length of fish j at annuli i. Using this method, growth data was available as far back as 1984 for five-year-old fish caught in Grand Falls Flowage in 1989, 1985 for fish caught in Woodland Flowage in 1990, and 1986 for fish caught in Big Lake in 1991.

Age 1 through age 5 smallmouth bass were tested separately for each lake to quantify variability in growth between alewife and no alewife years. Statistical analysis was performed with the non-parametric Mann-Whitney U test (Sokal and Rohlf 1995). Parametric statistical methods were not applicable to the growth data because growth at age t was autocorrelated with growth at age t-1. That is, smallmouth bass that showed above average growth at one age were more likely to show above average growth at subsequent ages, violating the assumption of independence between data points. The Mann-Whitney U Test tests the sums of the ranked distributions to determine if the distributions are different and thus makes no assumptions concerning normal distributions.

2. DIET ANALYSIS

Lakes

Seven lakes were chosen to conduct the diet analysis portion of the project. These lakes ranged in size from approximately 5 to 40 km^2 (2 - 5.5 mi^2) in area and 3 to 12 meters (9-36 feet) in average depth (Table 2). All seven lakes are low production systems with mean summer Chlorophyll-*a* concentrations of 0.99 µg/L to 2.76 µg/L. Alewives have free access into Cathance, Meddybemps and Gardner Lakes via Denil (Gardner and Meddybemps) or Alaskan steep-pass (Cathance and Meddybemps) fish ladders. Alewives are stocked into Woodland Flowage up to a density of 6 fish per acre by the Department of Fisheries Oceans Canada. Alewives have been excluded from Grand Falls Flowage and Big Lake since 1990 and Woodland Flowage between 1995 and 2000. Alewife harvests currently occur at Gardner Lake only. Alewives have been harvested from Meddybemps Lake and the St. Croix River at Milltown Dam in the past. Non-alewife lakes were all located within the St. Croix River watershed and constituted the two smallest and the largest lakes in the study. Although they will not be considered here, it is of note that Big Lake and Grand Falls Flowage have breeding populations of landlocked alewives, as does East Grand Lake on the east branch of the St. Croix River. An unknown number of landlocked alewives move downstream through Woodland Flowage

annually but they are not believed to spawn there because Woodland Flowage lacks appropriate over-winter habitat (M. Smith, Maine IFW, personal communication).

Table 2: Characteristics of the primary study lakes from which diet samples were collected. na = no data available. All total phosphorous (TP) measurements were below detection levels in Cathance Lake and the temperature logger in Pocumcus Lake was lost. ALE = anadromous alewife, Chl-a = Chlorophyll-*a*

Lake	Watershed	ALE	Area (km^2)	Mean Depth (m)	Summer Chl-*a* (µg/L)	TP (mg/L)	2005 June – Oct. average water temp. (^oC)
Cathance Lake	Dennys	Yes	11.76	7.3	0.99	na	20.9
Meddybemps Lake	Dennys	Yes	27.38	4.3	2.76	0.007	20.8
Gardner Lake	East Machias	Yes	15.05	12.2	2.38	0.005	20.3
Woodland Flowage	St. Croix	Yes	4.86	4.6	2.07	0.006	21.8
Big Lake	St. Croix	No	41.70	4	2.11	0.006	21.5
Grand Falls Flowage	St. Croix	No	27.09	3	2.53	0.006	21.8
Pocumcus Lake	St. Croix	No	8.95	7.6	2.11	0.007	na

Sample collection: Adult alewife diet habits

The objective of Hypothesis 2a was to obtain current information on the food habits of alewives. In particular this objective addressed the concern that young-of-year smallmouth bass were prey of adult alewives. Alewives were captured from Cathance, Meddybemps, Gardner and Woodland with trammel nets (1.8m tall, 30.5m long, 2.54 cm interior mesh, 30.4 cm outer mesh), an entanglement gear. Nets were set three times after dark; sets ran perpendicular to shore starting at the one meter depth contour and soak times were approximately 30 minutes. Fishing for adult alewives occurred between June 25 and July 22, 2005. Each lake was fished at least four nights, with the exception of Cathance Lake, which was only fished twice. No adult alewives were collected from Cathance Lake.

Fish processing included collecting standard measurements and a diet sample from captured alewives. Length measurements were taken to the nearest mm and weight measurements to the nearest gram. Diet samples were collected via gastric lavage (Hartleb and Moring 1995), whereby a 1.25 cm diameter tygon tube connected to a garden sprayer was inserted into the gut of the alewife; water was pumped into the gut, flushing the gut contents into an 80 µm brass sieve. Diet contents were then washed into a 236.5 ml (8 oz.) plastic cup and preserved in a solution of 80% alcohol, 15% water and 5% polyethylene glycol (F13) (Warmington et al. 2000). Within 24-36 hours, samples were concentrated into a 90 ml plastic vial with 70% ethanol for long-term storage. Fifteen alewives were sacrificed and their stomachs removed to verify the effectiveness of the gastric lavage. In all cases, gastric lavage cleared the foregut but left the contents of the hind gut largely intact. Hind gut contents were usually a single concentrated pellet of mostly digested material. Most of this material was not identifiable so the gastric lavage protocol was considered effective with the stipulation that only the foregut was being sampled.

Diet contents were identified to family when possible, and in most cases all organisms were counted. In cases where zooplankton were too numerous to count individually, three 2 ml subsamples were taken, all organisms of the focal taxa were counted and the results averaged.

The number of organisms was estimated by scaling the average number of organisms per 2 ml subsample up to the volume of fluid in the container prior to subsampling. Five representative diet items per fish from each category of diet items identified were measured to the nearest 0.01 mm with a 10x optical micrometer. Lengths of diet items were used to calculate an average length of prey ingested for each category of diet item identified.

Sample collection: Young-of-year alewife and smallmouth bass diet habits

The objective of Hypothesis 2b was to obtain current information on the food habits of young-of-year alewives and smallmouth bass. In particular this objective addressed the concern that YOY alewives were competing with YOY smallmouth bass for the same prey resources. Alewife and smallmouth bass young-of-year were fished from all lakes using a beach seine (1.2 m tall, 15.25 m long, 6.4 mm mesh). Lakes were sampled every two to three weeks between July 27 and September 9 during daylight hours. Samplings were subdivided into sample periods where July 27- Aug. 20 corresponded to sample period 1 or early, Aug. 21 – Sept. 6 corresponded roughly to sample period 2 or mid-summer, and Sept. 7 – Sept. 9 corresponded to sample period 3 or late summer. The goal of any single sampling date was to collect as many young-of-year alewives and young-of-year smallmouth bass as possible, up to 25 individuals. Twenty-five organisms provide an optimal balance between effort and diet resolution, especially for opportunistic species like *Micropterus spp.* (Carpenter and Kitchell 1993). Sampling ended when either the target number of individuals was reached or six hours had been spent at the lake.

Fish processing included lethal sampling of alewife and smallmouth bass young-of-year in the field; subsequent data collection occurred in the lab. Fish caught in the field were immediately preserved in F13 preservative solution. Before being transferred to 70% ethanol for long term storage, fish were removed from the preservative, measured to the nearest mm, and weighed to the nearest 0.1 g. The body cavity of the fish was opened and the stomach excised and rinsed into a 2 ml vial containing 70% ethanol.

Diet contents were identified to family whenever possible and all organisms in a sample were counted. Five representative diet items from each category of diet item identified were measured to the nearest 0.01 mm with a 10x optical micrometer. Lengths of diet items were used to calculate an average length of prey ingested for each category of diet item identified.

Auxiliary data collection: Water temperature, Total phosphorus and Chlorophyll-*a*

Additional data was collected from each lake at three set sampling sites. A continuous record of water temperature was collected via a submersible thermal logger (Hobo Pendant) deployed at 1-2 m depth. Water samples collected at the three set sampling sites were tested for Total phosphorous (TP) and Chlorophyll-*a* (Chl-*a*). TP and Chl-*a* samples were collected via Van Dorn bottle from mid-depth at each sample site (3 m at 6 m deep sites or 1.5 m at two 3 m deep sites) and stored in separate polypropylene bottles (1 liter for Chl-a and 0.5 liter for TP). Water samples were kept in the dark and at approximately 4^{o}C until processed by the New Brunswick Department of the Environment Analytical Services Laboratory located in Fredericton, NB, Canada. Temperature/dissolved oxygen profiles (1 m resolution) were also collected at each of the three set sampling sites with a YSI-85 temperature/dissolved oxygen meter.

Analyses

Diet data were expressed as a percent of the index of relative importance (%IRI). The index of relative importance combines frequency of occurrence as percent occurrence (%O), diet category weight as percent weight (%W) and diet category numerical occurrence as percent of number (%N). Calculations are as follows (from Liao et al. 2002):

$$(8) \qquad \%IRI_i = 100 \text{ x } \frac{IRI_i}{\sum_{j=1}^{n} IRI_j}$$

and

$$(9) \qquad IRI_i = \%O_i \times (\%W_i + \%N_i)$$

For this analysis, percent length ($\%L_i$) was substituted for $\%W_i$ because many diet items were either too small or too degraded to weigh. Literature length to dry mass conversion formulas were not available for many of the families of invertebrates identified from the fish diets. Therefore, the following IRI modification was substituted for the above:

$$(10) \qquad IRI_i = \%O_i \text{ x } (\%L_i + \%N_i)$$

where $\%L_i$ was defined as:

$$(11) \qquad \%L_i = 100 \text{ x } \frac{L_i}{\sum_{j=1}^{n} L_j}$$

where L_i is the total length (mm) of prey in a comparison unit. L_i was calculated by multiplying the average length of measured diet items from a fish by the total number of counted diet items in that same fish. Other parameters include:

$$(12) \qquad \%N_i = 100 \text{ x } \frac{N_i}{\sum_{j=1}^{n} N_j}$$

$$(13) \qquad \%O_i = 100 \text{ x } \frac{O_i}{\sum_{j=1}^{n} O_j}$$

where n is the total number of prey taxa in a comparison unit. N_i is the number of prey i in a comparison unit. O_i is the number of predator stomachs containing prey i in a comparison unit. IRI_i is the value of IRI for prey i in a comparison unit (Liao et al. 2002).

Competition between young-of-year alewife and young-of-year smallmouth bass was assessed by calculating Schoener's Index (Schoener 1970). Schoener's Index compares

differences in the proportions of prey comparison units and sums across all units to arrive at an index value that is a measure of diet overlap between two species or groups of organisms. Schoener's Index is calculated as:

$$(14) \qquad \alpha = 1 - 0.5 \times \left(\sum_{i=1}^{n} \left| P_{xi} - P_{yi} \right| \right)$$

where P_{xi} is the proportion of food item (taxa) i in the diet of young-of-year alewives, P_{yi} is the proportion of food item i in the diet of young-of-year smallmouth bass and n is the number of prey categories (Kahilainen and Ostbye 2006). Here Schoener's Index is expressed as a percentage (i.e., α x 100 = %α) and IRI values were substituted for P. A value of 0% indicates no diet overlap and a value of 100% indicates complete diet overlap. It is assumed that fish diet overlap must be greater than or equal to 60% in order to be biologically significant, a value that has been widely accepted in scientific literature (Zaret and Rand 1971, Wallace 1981, Willis et al. 2002, Kahilainen and Ostbye 2006, Kahl and Radke 2006).

In cases where significant (≥ 60%) diet overlap was detected, the average length of diet items consumed (averaged per fish for a give period of time) was used to further assess the degree of diet overlap. Alewives and smallmouth bass have distinctly different mouth sizes and thus the potential to eat different sizes of prey at the same body length. This difference may translate into consumption of different species of the same family or different size classes of the same species (Labropoulou and Eleftheriou 1997). Where warranted, i.e. when %α ≥ 60%, a 2x2 ANOVA analysis was used look for differences in the size of diet items ingested between species across sample periods.

3. BASS TOURNAMENT RESULTS ANALYSIS

Lakes

Thirteen lakes were used to assess how alewives affected tournament bass fishing. These lakes range in size from approximately 5 to 40 square kilometers (Table 3).

Sample collection: Tournament results

Tournament data were provided by the Maine Blade Runners Bass Club and the New Brunswick Sport Fishing Association. Data consisted of entries for individual teams collected at weigh-in, i.e. the total weight of a bag of live smallmouth bass and the number of fish in that bag. Fishing tournaments generally were eight hours in length, starting at approximately 6 AM and ending around 2-3 PM. Most tournament entries were capped at a five fish bag limit, although six fish per bag was an infrequent occurrence in 2002 in New Brunswick and 2003 in Maine. Without additional metadata to determine if these occurrences were erroneous (i.e. different rules for tournaments in question), cases of more than five landed smallmouth bass were excluded from the analysis to match tournament guidelines used in 2005 in both Maine and New Brunswick. Records of tournaments in New Brunswick started in 2002 whereas records for Maine tournaments began in 2003.

Table 3: Characteristics of lakes used in the bass tournament entry analysis. Years = years for which tournament data were available. Alewives were absent from four lakes.

Lake	State/ Prov.	Watershed	Area (km^2)	Years	Tournaments	Alewife present
Harvey	NB	Magaguadavic	7.0	2002-2005	5	No
Magaguadavic	NB	Magaguadavic	27.4	2002-2005	6	No
Big	ME	St. Croix	41.7	2003-2005	3	No
Grand Falls	ME	St. Croix	27.1	2004, 2005	3	No
Boyden	ME	Little River	7.1	2003-2005	3	Yes
Crawford	ME	E. Machias	7.6	2003-2005	4	Yes
Gardner	ME	E. Machias	15.1	2003-2005	9	Yes
Hadley	ME	E. Machias	7.2	2005	1	Yes
Mactaquac	NB	Saint John	~ 83.5	2002-2005	4	Yes
Meddybemps	ME	Dennys	27.38	2005	1	Yes
Pocomoonshine	ME	E. Machias	10.3	2003-2005	8	Yes
Utopia	NB	Magaguadavic	14.1	2002-2005	7	Yes

Analyses

Lakes were split into categories of with and without alewives. Analysis consisted of a full factorial ANOVA with year, alewife category and an interaction term. Dependent variables were annual average weight per team and annual average weight per smallmouth bass.

4. ALEWIFE GENETICS ANALYSIS

Bentzen and Paterson (2005) examined the genetics of landlocked and anadromous alewife populations in the St. Croix watershed in a separate study for this project. Their findings are summarized in this report.

RESULTS

1. EXISTING AGENCY DATA ANALYSIS

Young-of-year smallmouth bass percent relative weight (%Wr), Fulton condition (K_b) and length were not systematically different in lakes with alewives vs. lakes without alewives (Table 4A). Two-way ANOVAs were performed on lakes grouped into with and without alewife categories and years in Woodland Flowage at different alewife stocking densities. ANOVA results for the lakes analysis indicated that variability between lakes was a significant source of variation in young-of-year smallmouth bass %Wr ($p < 0.00$), length ($p < 0.00$) and K_b ($p < 0.00$) values (Table 4A). Alewife presence-absence was not a significant source of variation for the condition measures or length ($p > 0.5$ in all cases). Among lakes with alewives, Cathance young-of-year smallmouth bass had the highest condition, but young-of-year smallmouth bass in Meddybemps Lake were longest by the end of the first growing season (Fig. 4). Among lakes without alewives, Grand Falls Flowage young-of-year smallmouth bass had the highest

condition, but young-of-year smallmouth bass from Green Lake were longest by the end of the first growing season.

Table 4: Two-way ANOVA results for condition indices and length data for young-of-year smallmouth bass. For all models, the presence or absence of alewives was not a significant factor. Lake was a significant factor for the Among Lakes model (A) and year was a significant factor for the Among Years in Woodland Flowage model (B). %Wr = Percent relative weight, K_b = Fulton's Condition Factor. Alewife present lakes $n = 3$, alewife absent lakes $n = 6$; alewife present years $n = 6$, alewife absent years $n = 3$.

A. Among Lakes

	Effect	Sum-of-Squares	df	Mean Square	F-ratio	p
%Wr Arcsin-Sqrt	Alewife present/ absent	0.00	1	0.00	0.00	1.00
	Lake	0.04	7	0.01	6.5	< 0.00
	Error	0.06	76	0.00		
Length	Alewife present/ absent	3.31	1	3.31	0.15	0.70
	Lake	950.65	7	135.8	6.16	< 0.00
	Error	1676.64	76	22.06		
K_b Arcsin-Sqrt	Alewife present/ absent	0.00	1	0.00	0.015	0.90
	Lake	0.08	7	0.01	6.457	< 0.00
	Error	0.13	76	0.00		

B. Among Years - Woodland Flowage

	Effect	Sum-of-Squares	df	Mean Square	F-ratio	p
%Wr Arcsin-Sqrt	Num. ALE spawners	0.00	1	0.01	0.37	0.55
	Year	0.20	7	0.03	13.11	< 0.00
	Error	0.73	339	0.00		
Length	Num. ALE spawners	161.12	1	161.12	2.34	0.13
	Year	544.54	7	780.66	11.36	< 0.00
	Error	23305.66	339	68.75		
K_b Arcsin-Sqrt	Num. ALE spawners	0.01	1	0.01	2.52	0.11
	Year	0.39	7	0.06	12.56	< 0.00
	Error	1.52	339	0.00		

Young-of-year smallmouth bass percent relative weight (%Wr), Fulton condition (K_b) and length were not systematically different in years with alewives vs. years without alewives for Woodland Flowage ($p > 0.1$ in all cases; Table 4B). However, annual variation was a significant source of variability for length and condition indices ($p < 0.00$ in all cases; Table 4B). For years when alewives were present, 1994 (at 717 alewife per acre) had the lowest condition, but was similar in value to 2003 (at 15 alewife per acre) (Fig. 5). Condition was highest in 2002, followed closely by 2005. Young-of-year smallmouth bass achieved their longest average growth in 2001 and 2003. For lakes without alewives, condition was very similar among years but highest in 1995. Length was also greatest in 1995.

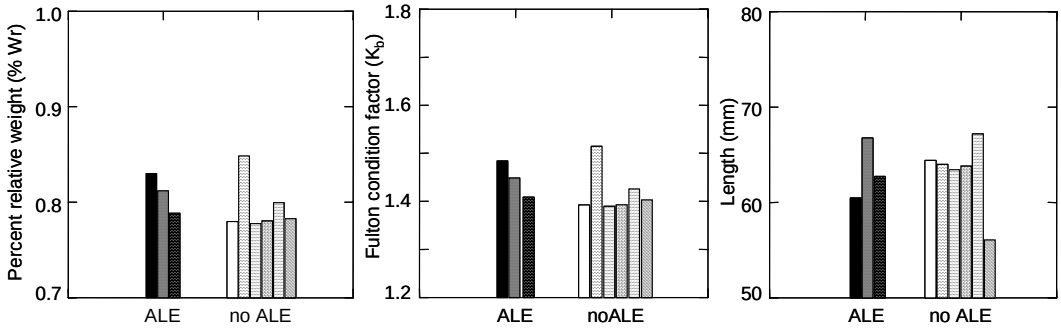


Figure 4. Bar graph depicting among lake data for young-of-year smallmouth bass. Each bar is the average of available annual data from a lake. Alewife (ALE) vs. no alewife (no ALE) was not a statistically significant designation. Among lake variation was statistically significant for all three models. ALE lakes are, from left to right, Cathance, Meddybemps, Pocomoonshine. No ALE lakes are, from left to right, Big, Grand Falls, Beech Hill, Branch, Green, West Grand.

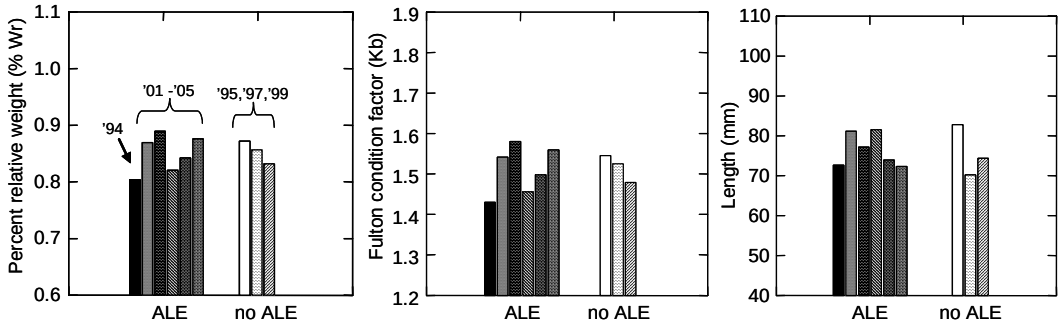


Figure 5. Bar graph depicting among year data for Woodland Flowage young-of-year smallmouth bass. Each bar is the average of individual smallmouth bass from a given year. Whether alewife (ALE) were present in a year was not statistically significant. Among year variation was statistically significant for all three models. ALE years are, from left to right, 1994, 2001, and 2005. No ALE years are, from left to right, 1995, 1997, and 1999.

Estimates of smallmouth bass growth, based on the scale back-calculation method, indicated that growth generally was not retarded by the presence of alewives. Significant differences in smallmouth bass growth in years with alewives vs. years without alewives varied between lakes (Fig. 6). For Big Lake, age 2 smallmouth bass grew significantly more when alewives were present and age 3 fish showed a trend towards faster growth with alewives present ($p < 0.07$). For Grand Falls Flowage, age 1 and age 3 smallmouth bass grew significantly more, and for Woodland Flowage, age 2, age 3 and age 4 smallmouth bass grew significantly more in years when alewives were present. Age 5 smallmouth bass showed signs of slower growth in years when alewives were present in Big Lake and Grand Falls Flowage, but those differences were not statistically significant.

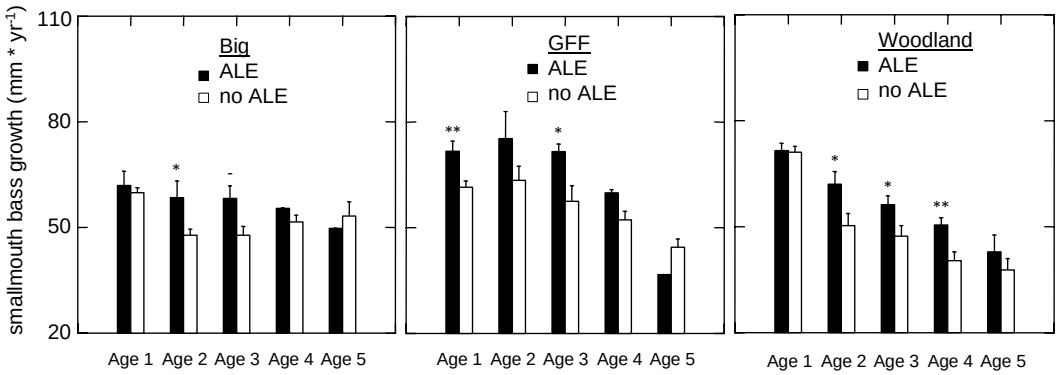


Figure 6. Bar graph depicting smallmouth bass growth by year in millimeters for Big Lake, Grand Falls Flowage (GFF) and Woodland Flowage comparing years with alewives present (ALE) and years with alewives absent (no ALE). Error bars are standard deviations (± 1SD). Results of the non-parametric Mann-Whitney U test are also shown on the figure; comparisons with ** were statistically significant at the $p < 0.01$ level, * = $p < 0.05$, - = $p < 0.1$. Sample size in years of data for an age class: Big = Big Lake, GFF = Grand Falls Flowage, Woodland = Woodland Flowage. Age 1 *n*: Big = 5 (ALE), 8 (no ALE); GFF = 7 (ALE), 10 (no ALE); Woodland = 12 (ALE), 6 (no ALE). Age 2 *n*: Big = 4 (ALE), 10 (no ALE); GFF = 6 (ALE), 11 (no ALE); Woodland = 11(ALE), 6 (no ALE). Age 3 *n*: Big = 3 (ALE), 11 (no ALE); GFF = 4 (ALE), 12 (no ALE); Woodland = 10 (ALE), 6 (no ALE). Age 4 *n*: Big = 2 (ALE), 12 (no ALE); GFF = 2 (ALE), 12 (no ALE); Woodland = 7 (ALE), 5 (no ALE). Age 5 *n*: Big = 1 (ALE), 6 (no ALE); GFF = 1 (ALE), 6 (no ALE); Woodland = 5 (ALE), 3 (no ALE).

2a. ADULT ALEWIFE DIET HABITS

Adult alewives were collected from Gardner (*n* = 38) and Meddybemps (*n* = 21) Lakes and Woodland Flowage (*n* = 11) in 2005. No adult alewives were collected from Cathance Lake. Most alewives were caught in the cove nearest the lake outlet, presumably the point of access to the lake, but alewives were also collected in more distant coves. Most alewives were collected in less than ten feet of water at night, removed from the trammel net alive and returned to the lake in swimming condition after collection of measurements and stomach contents. The majority of mortality was caused by snapping turtles consuming parts of alewives while nets were soaking.

Adult alewives consumed a range of diet items once in freshwater. In Meddybemps Lake and Woodland Flowage zooplankton made up the majority of diet items, whereas in Gardner Lake mayfly larvae (ephemeroptera) were the most important single identifiable diet item (Fig. 7).

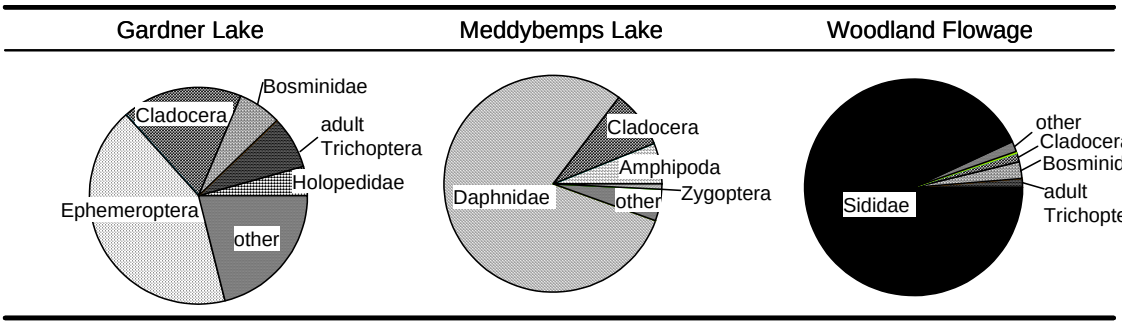


Figure 7. Pie graphs of adult alewife diet proportions by lake. Proportions are %IRI which combines diet item length, frequency of occurrence and numerical abundance data into one measure. Sample sizes in number of fish: Gardner *n* = 38, Meddybemps *n* = 21, Woodland *n* = 11.

Fish made up a very small proportion of the diet of adult alewives collected. In general, fish prey made up less than 0.15% of the diet by %IRI, which combines % frequency of occurrence, % numerical abundance and % size (length) (Fig. 8). In Meddybemps Lake %IRI was 0.02% and no fish were found in the stomachs of adult alewives collected in Woodland Flowage. In other words, of 70 adult alewives sampled from three lakes 7, or 10%, had fish in their guts. However, when the total number of diet items consumed and the contribution of fish to the length of all items eaten were taken into account, fish were less than 1% of the diet. Most fish prey that were found in alewife stomachs had the general body characteristics of either larval stage rainbow smelt (*Osmerus mordax*) or larval stage alewives.

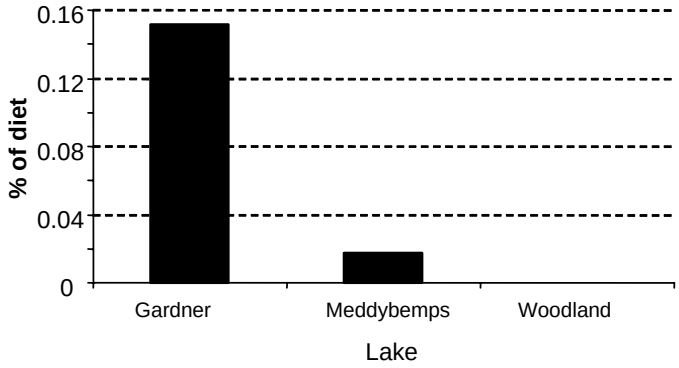


Figure 8. Percent of adult alewife diets that consisted of fish. Proportions are %IRI which combines diet item length, frequency of occurrence and numerical abundance data into one measure. At most, fish consisted of less than 0.16% of adult alewife diets. All but one of seven fish found in diets resembled larval alewife or rainbow smelt.

2b. YOUNG-OF-YEAR ALEWIFE AND SMALLMOUTH BASS DIET HABITS

Young-of-year (YOY) fishes were caught in mixed schools in less than four feet of water where both alewives and smallmouth were present. Based on this, fish were presumably feeding in the same habitat alongside each other. However, there was considerable variation in young-of-year diet by lake. Zooplankton were by far the most often found diet item in all lakes, but YOY smallmouth bass diversified their diets with other invertebrates, e.g. mayfly larvae, midge larvae, and adults of these invertebrates. In Gardner and Meddybemps Lakes, members of the suborder

Cladocera made up the most abundant diet items for both alewives and smallmouth bass. In Gardner, YOY alewife diets were dominated by Bosminidae, followed by Chydoridae, both small zooplankters in the suborder Cladocera (Fig. 9). YOY smallmouth bass in Gardner ate mostly Sididae, a larger zooplankter that tends to concentrate in and around aquatic vegetation, and mayfly larvae. In Cathance Lake, Chaoboridae (phantom midge), fly larvae that prey on zooplankton, were the most important diet item for YOY alewife, whereas YOY smallmouth bass concentrated on Sididae, Polyphemus, a small predatory zooplankter, and Chydoridae (Fig. 9). In Woodland Flowage, adult Diptera, generally Simulidae (black flies), Trichoptera (generally net-spinning caddis flies) and chironomids made up the majority of the diets of both YOY alewife and YOY smallmouth bass (Fig. 9). However, 50% of YOY alewife diet consisted of Simulidae, whereas the same organisms made up only a small percentage of the YOY smallmouth bass diet. In Meddybemps the majority of YOY alewife and smallmouth bass diets consisted of Sididae.

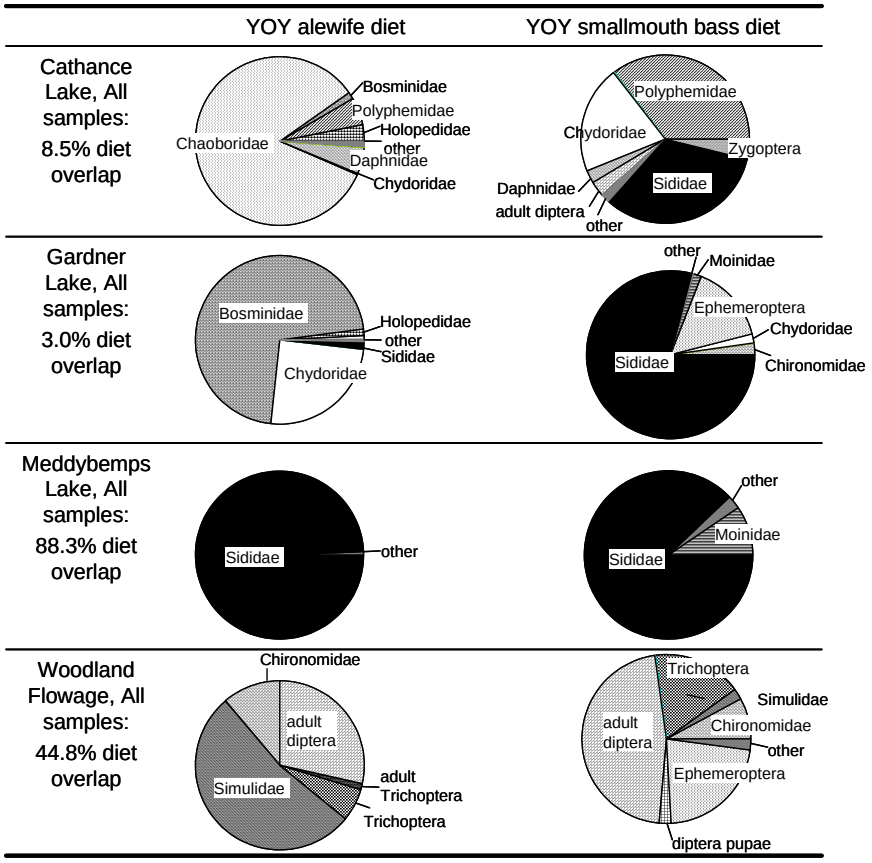


Figure 9. Pie graphs of young-of-year alewife and young-of-year smallmouth bass diet proportions by lake. Proportions are %IRI which combines diet item length, frequency of occurrence and numerical abundance data into one measure. Values for Schoener's Index of diet overlap are given in the left hand column. Diet overlap >60% is considered biologically significant. Sample size in number of fish: Cathance - YOY alewife (ALE) n = 50, YOY smallmouth bass (SMB) n = 19; Gardner - YOY ALE n = 35, YOY SMB n = 17; Meddybemps - YOY ALE n = 58, YOY SMB n = 35; Woodland - YOY ALE n = 7, YOY SMB n = 36.

Of the four lakes sampled with sympatric populations of smallmouth bass and alewives, only Meddybemps Lake demonstrated significant diet overlap. For the early summer and mid summer sample periods and for all diets, the combined Schoener's Index exceeded the 60% threshold needed for a biologically relevant interaction (Table 5). At 78% to 90% diet overlap, it is possible that young-of-year alewives and young-of-year smallmouth bass were competing for the same food resources. Young-of-year in Cathance and Gardner Lakes had a less than 15% diet overlap by sampling period and overall (Table 5). Diet overlap was higher in Woodland Flowage with a maximum of 45% when all samples were considered, however this was still below the 60% threshold.

Both smallmouth bass and alewives in Meddybemps Lake ate zooplankton in the family Sididae almost exclusively, creating a basis for the high degree of diet overlap observed in that lake (Fig. 9). There are at least four species of Sididae in New England lakes, including the genera Latona, Diaphanosoma and Sida that have been found in the Downeast region (U.S. Environmental Protection Agency 1994). These species vary by body size and depth distribution in lakes (Dole-Olivier et al. 2000, Great Lakes Environmental Research Lab 2004). Due to the partially digested state of most zooplankton found in fish diets, positively identifying Sididae to genus was generally not possible. However, it was possible to test for differences in Sididae carapace length in the diets of smallmouth bass and alewives. The model y = constant + sample period + species + interaction + error was significant for all model terms (Table 6). That is, there was a significant difference in the length of Sididae eaten by the two species, along with a significant difference in the length of Sidiae ingested during the two sampling periods. During the early summer period, young-of-year smallmouth bass and young-of-year alewives consumed different sized Sididae. By mid summer, young-of-year alewives were eating Sididae the same size as those being eaten by young-of-year smallmouth bass (Fig. 10).

Table 5: Schoener's Index of diet overlap values for YOY alewives and YOY smallmouth bass presented by sample period and all sample periods combined. Schoener's Index was calculated with %IRI, which combines diet item length, frequency of occurrence and numerical abundance data into one measure. Schoener's Index is an approximation of expected competition between species where a value > 60% indicates biologically significant diet overlap. Meddybemps Lake was the only lake demonstrating significant diet overlap and thus a likelihood for competition. - = insufficient data to calculate diet overlap index. Sample sizes by period available in a separate data report. Combined uses all available data, including data not included in individual periods because no members of the opposing species were available to complete the diet overlap calculation for that period.

Lake	Early period	Middle period	Late period	Combined
Cathance	6.6%	14.6%	-	8.5%
Gardner	-	3.4%	2.4%	3.0%
Meddybemps	-	89.9%	78.1%	88.3%
Woodland	-	39.0%	-	44.8%

The size of Sididae ingested by smallmouth bass in Meddybemps Lake did not change between early summer and mid summer, but the size of Sididae ingested by alewives increased over the same period. Competition for zooplankton in the mid summer period was significant between young-of-year alewives and young-of-year smallmouth bass.

Table 6: Two-way ANOVA models with interaction results for assessing variability in the length of Sididae ingested by YOY alewife and YOY smallmouth bass in Meddybemps Lake. All terms in the model were significant. Ingested Sididae length differed between species, increased between sampling periods and those changes were not independent, i.e., length of ingested Sididae increased through time.

Source	Sum-of-Squares	Degrees of Freedom	Mean-Square	F-ratio	P value
Sampling period	0.43	1	0.43	31.18	0.00
Species	0.58	1	0.58	41.59	0.00
Sampling period-species interaction	1.04	1	1.04	74.93	0.00
Error	0.90	65	0.01		

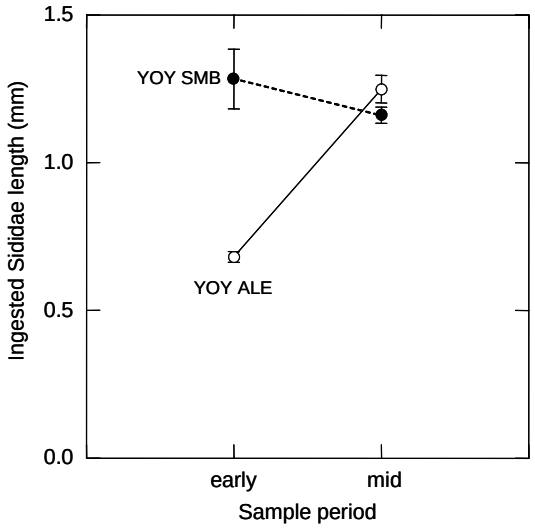


Figure 10. Interaction plot for ANOVA results in Table 6 for Meddybemps Lake. The length of Sididae ingested by YOY smallmouth bass did not change between sample periods early and mid. However the length of Sididae ingested by YOY alewife increased so that there was overlap by the mid summer sample period.

Since Meddybemps was the only lake in which smallmouth diets and alewife diets were ecologically similar, comparing Meddybemps smallmouth bass diets to lakes without alewives may provide an indication of what lakes would require close monitoring for potential alewife – smallmouth bass competitive interactions. In particular, diet overlap > 60% between Meddybemps Lake and the no alewife lakes (Big, Grand Falls, and Pocumcus) could indicate a greater possibility for competition between alewives and smallmouth bass in that lake if alewives were permitted passage into that portion of the drainage. This assumption is predicated on young-of-year alewives also showing a preference for Sididae in that new habitat. Bass in Pocumcus Lake showed 86.3% similarity in diet to bass in Meddybemps (Table 7). This suggests a chance of significant diet overlap between alewives and bass in Pocumcus. However, bass diet in Pocumcus also showed 84.3% similarity with bass diets in Gardner Lake, where bass and alewives did not show ecologically similar diets.

Table 7: Schoener's Index of diet overlap values for YOY smallmouth bass for all sample periods combined. Here Schoener's Index is used to assess diet similarity between populations of smallmouth bass, again using a value > 60% to indicate biologically significant diet similarity. Pocumcus Lake is the only lake where YOY smallmouth bass appear to have similar diet habits to Meddybemps Lake YOY smallmouth, which showed significant diet overlap with YOY alewife. Pocumcus YOY smallmouth could also demonstrate significant diet overlap with YOY alewife if anadromous stock were ever introduced to that lake. However, Pocumcus YOY smallmouth diets were also similar to Gardner smallmouth where no alewife – smallmouth diet overlap was demonstrated. Sample sizes as number of fish: Big = 49; Grand Falls = 32; Pocumcus = 34.

Lake	Big	Grand Falls	Pocumcus
Cathance	10.2%	37.5%	38.0%
Gardner	6.3%	61.9%	84.3%
Meddybemps	3.7%	46.1%	86.3%
Woodland	14.3%	26.3%	9.8%

The diet of YOY smallmouth bass in Big Lake in early summer consisted of Diptera pupae and Daphnidae; in mid summer Sididae, Ephemeroptera, Diptera adults, Chironomids made up most of the diet (Fig. 11). The diet of YOY smallmouth bass in Grand Falls Flowage in early summer consisted of mostly Polyphemidae and Diptera adults; in mid summer Sididae and Ephemeroptera made up most of the diet (Fig. 11).

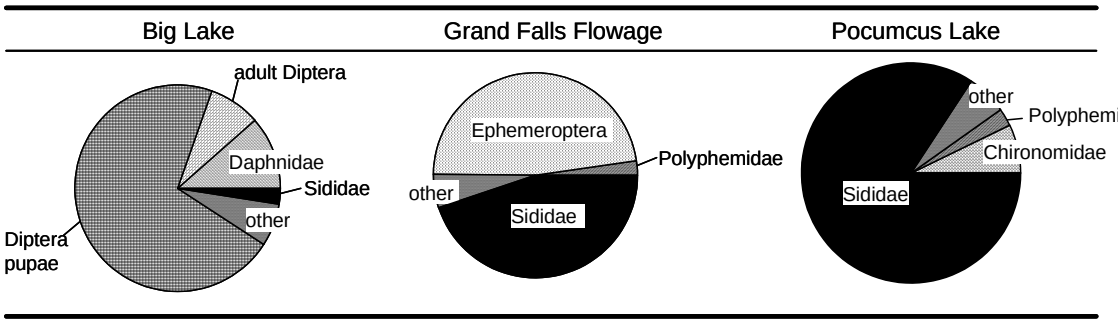


Figure 11: Pie graphs of YOY smallmouth bass diet proportions for lakes without alewives. Proportions are %IRI, which combines diet item length, frequency of occurrence and numerical abundance data into one measure. Sample sizes in number of fish: Big $n = 49$, Grand Falls $n = 32$, Pocumcus $n = 34$.

2. BASS TOURNAMENT RESULTS ANALYSIS

Analysis of smallmouth bass fishing tournament landings from New Brunswick and Maine did not indicate that a systematic difference in the weight of entries existed between lakes with alewives and lakes without alewives. Whether alewife were present or absent was not statistically important ($p < 0.05$) in the ANOVA models of either weight per team or weight per fish (Table 8). The year term was not significant in the weight per fish landed model, but showed a strong trend ($p = 0.055$) in the weight per team model. The landed weight per team was lower in 2005 than in the three previous years (Fig. 12). The interaction between year and whether

alewife were present or absent was not statistically significant in either the per fish or per team model.

Table 8: Results of two-way ANOVA with interaction models used to assess variability in bass tournament entries between lakes with and without alewife and between years. Neither model was significant, indicating that neither weight per team nor weight per fish differed statistically between years or lakes with and without alewives. There was a trend towards weight per team being significantly different between years ($p < 0.1$).

	Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Weight per team	Alewife present/ absent	2.50	1	2.50	0.92	0.34
	Year	22.89	3	7.63	2.82	0.06
	Alewife x Year	1.33	3	0.44	0.16	0.92
	Error	86.56	32	2.71		NS
Weight per fish	Alewife present/ absent	0.17	1	0.17	0.92	0.33
	Year	0.80	3	0.27	1.46	0.24
	Alewife x Year	0.06	3	0.02	0.11	0.95
	Error	5.86	32		0.18	

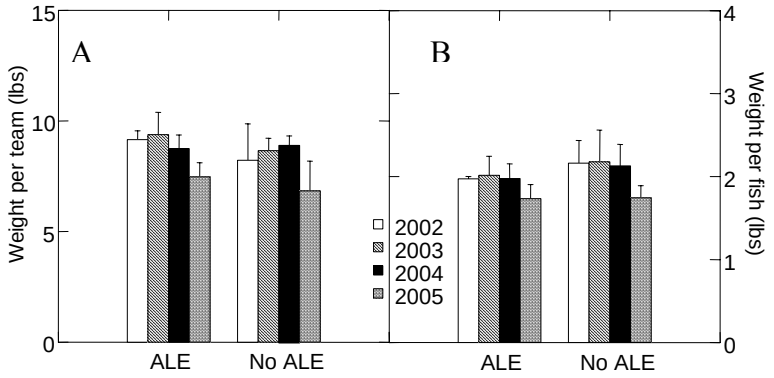


Figure 12. Bar graph depicting among year ANOVA results for (A) bass tournament entry weight per team and (B) average weight per fish. Neither lakes with alewives nor lakes without alewives nor years were significantly different in the two models. However, tournament weight per team did show a strong decline in 2005 in both ALE and no ALE lakes. Error bars are standard error (±1 SE). Sample sizes as number of lakes: 2002 ALE $n = 2$, no ALE $n = 3$; 2003 ALE $n = 6$, no ALE $n = 4$; 2004 ALE $n = 6$, no ALE $n = 4$; 2005 ALE $n = 8$, no ALE $n = 4$.

4. ALEWIFE GENETICS ANALYSIS

Alewives collected from Milltown Dam, Dennis Stream and the upper St. Croix drainage were used to determine the genetic relatedness among sub-populations of St. Croix alewives. Collected fish were presumed anadromous stock from the former two locations and landlocked stock from the latter. In addition, two distant anadromous stocks were used to test whether landlocked stocks were more closely related to St. Croix anadromous stocks or to more

geographically distant anadromous stocks. Landlocked alewives were found to be distantly related to all the anadromous stocks tested (Fig. 13). A variety of statistical tests confirmed that anadromous and landlocked populations of alewives in the St. Croix are genetically divergent (FST = 0.244). These results implied that very little, if any, interbreeding occurs between the two life history types. The results of assignment tests indicated that it should be possible to reliably identify the life history type of individual St. Croix alewives at any life history stage by genotyping them at five microsatellite loci. Significant genetic differences were observed between anadromous alewife populations in the St. Croix and anadromous populations in the LaHave and Gaspereau Rivers, as well as between the two anadromous St. Croix samples, Dennis Stream and Milltown. These results imply homing of alewives to their natal streams and, consequently, at least partial reproductive isolation between spawning runs, even at the level of tributaries within the St. Croix River. However, the degree of genetic differentiation between the two St. Croix samples was small (FST = 0.008), and needs to be evaluated further. For complete methods and results see Bentzen and Paterson (2005).

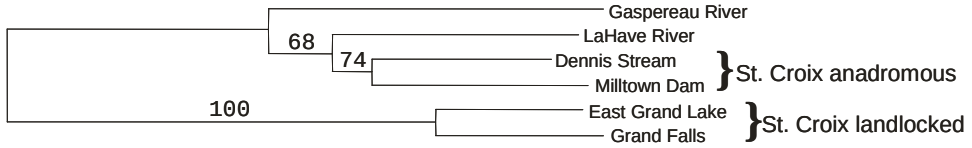


Figure 13. Neighbor-joining tree based on Cavalli-Sforza and Edwards chord distance between population samples analyzed using micro-satellite markers. Numbers indicate statistical support (% certainty of difference) for particular groupings. From Bentzen and Paterson (2005).

DISCUSSION

Competition between fish species can manifest as either interference competition or exploitation competition. Interference competition usually involves interaction between two species where the more aggressive species displaces the weaker species. Exploitation competition is competition for a common limiting resource (Matthews 1998). The degree of competition between two species (inter-specific competition), which can be either interference or exploitation competition, is often determined by the niche overlap between those species (Werner and Gilliam 1984, Matthews 1998). That is, two species with very similar prey preferences that occupy similar habitats are more likely to compete, usually to the detriment of the "weaker" species. For example, smallmouth bass and largemouth bass are the subjects of numerous competition studies because of their close evolutionary and ecological relationship (Near et al. 2003). In most cases, smallmouth bass exhibit resource segregation from inter-specific competitors, i.e., rock bass, largemouth bass, and bluegill, through habitat segregation and differential food choice from an early age (George and Hadley 1979, however see Sowa and Rabeni 1995, Olson et al. 2003).

The effects of competition are ultimately measured in terms of fitness of the focal population, that is, population growth through time in the presence of the competitor. Positive population growth despite the presence of a competitor means deleterious effects of any competition are minimal; population decline translates to probable deleterious effects associated

with the competitive interaction. Assessing the condition of the focal population at a vulnerable or critical life stage also may provide information regarding any effects of inter-specific competition. Ideally, studies tracking changes in population fitness should take several years (greater than 1 generation (Magnuson 1990)) to account for short-term cycles and random population fluctuations. Studies of this length are often cost prohibitive and population estimates are data hungry and difficult to execute. Other measures like fish condition (length-weight relationships) are often used to gauge the degree of competitive interactions. Snapshot measures of interaction for resources through diet comparisons can also provide evidence of competition, however some measure demonstrating lower fitness of the focal species should be provided to demonstrate a deleterious effect of the interaction. For example, significant overlap in diet still may not lead to negative population effects if food is available in excess of population requirements.

EXISTING AGENCY DATA

Pre-existing data collected by the Maine Department of Inland Fisheries and Wildlife showed differences in young-of-year (YOY) smallmouth bass condition between lakes and between years in Woodland Flowage. However, there was no systematic difference in YOY smallmouth bass condition based on the presence or absence of anadromous alewives, nor was there an interaction between lake or year and alewife presence-absence that might point to a more complicated relationship between lake groupings.

Variability between sampling units was high. Variation within alewife groups between lakes and between years within lakes far exceeded any systematic difference between alewife and no-alewife groups. This may be due in part to order of magnitude fluctuations in alewife abundance between the years investigated, i.e., there may have been far fewer alewives in Woodland Flowage between 2001 and 2005 than there were prior to 1995. However, both the years in which bass showed the best condition and the year in which bass showed the poorest condition were years in which alewives were present. Indeed, the lowest occurrences in smallmouth bass condition occurred in 1994 and 2003 when alewives were extremely abundant and the run was extremely depressed, respectively.

High variability between lakes is not uncommon in comparative studies. In the only other published study conducted explicitly on anadromous alewives and smallmouth bass, evidence of competition between YOY anadromous alewives and YOY smallmouth bass was found in Mactaquac Lake, a large New Brunswick reservoir, but not in Oromocto Lake, a nearby unimpounded lake (Hanson and Curry 2005). Threadfin shad (*Dorosoma petenense*), another clupeid, competed with bluegill for zooplankton prey in an Ohio reservoir, creating a cascading effect that ultimately reduced largemouth bass growth; in a second lake there was no apparent competition between bluegill and threadfin shad and no deleterious effects were noted further up the food chain (Devries et al. 1991). High inter-lake variability is noteworthy here because much of the approach to alewife management in the St. Croix is based upon the unsubstantiated assumption that observations from Spednic Lake are widely applicable to other lakes with sympatric alewife and smallmouth bass populations.

GROWTH

Growth of one year and older smallmouth bass was not systematically lower across ages or across lakes for Big Lake or Grand Falls and Woodland Flowages. Conversely, growth was significantly higher for smallmouth bass in the presence of alewives for at least one age interval in all three lakes.

Piscivorous fish growth and age at maturity are positively related to the availability of fish prey (Werner and Gilliam 1984); *Mircopterus spp*. show higher growth when fish prey of appropriate size is available early in the first year (Ludsin and DeVries 1997, Olson et al. 1998). YOY smallmouth bass growth rates have increased in Lake Erie since the introduction of round goby, which are now an ultra-abundant source of fish prey (Steinhart et al. 2004). In southeastern Massachusetts, alewife were a dominant prey item of 15-30 cm largemouth bass in trophy largemouth bass lakes (Yako et al. 2000). However, size structured interactions can retard fish predator growth in the first year (Olson et al. 1995, Bystrom et al. 1998). When prey and predator compete for the same food resources at small sizes, prey species can prevent predators from exceeding the gape limitation that prevents predators from eating that prey. This relationship has been explored thoroughly with largemouth bass and bluegill where competition for zooplankton and benthic invertebrates delays bass switching to fish prey. A similar size structured interaction may have driven the resource competition observed between smallmouth bass and alewife in Mactaquac Lake, but likely was not present in Oromocto Lake where a larger size range of YOY alewife facilitated piscivory by YOY smallmouth bass (Hanson and Curry 2005).

ADULT ALEWIFE DIET HABITS

Diets of adult alewives indicated that fish prey make up an extremely small percentage of the diet. These results agree with earlier results collected by Maine IFW for Cathance and other lakes in the Region C (Jordan 1990). More recent studies also corroborate the diversity and content of adult alewife diets (Kircheis et al. 2002). Most published accounts do not recognize the feeding of adult alewives in lakes and assume that, like many migratory spawners, alewives fast while migrating and leave the freshwater environment shortly after spawning (Havey 1961, Tyus 1974).

Landlocked alewives in the Great Lakes, where they are an invasive species renowned for their direct and indirect negative impacts on native fishes (for a review see Madenjian et al. 2002), have been shown to eat larval yellow perch (*Perca flavicens*) (Kohler and Ney 1980). However there are a number of distinctions between landlocked and anadromous alewives, particularly the duration of time that they inhabit freshwater. Both landlocked and anadromous alewives are very fecund: a gravid female will contain between 60,000 and 100,000 eggs, many of which become prey for other lake organisms. The survivors of a year class of anadromous alewives are present in freshwater during relatively short windows of time twice in four years: for up to 4-5 months in their first year of life and for several weeks 4-6 years later when they return to spawn. Landlocked alewives, by definition, are permanent freshwater residents and thus layer successive year classes over each other in a lake, compounding their ecological effects. Applying the ecological impacts of landlocked alewives to anadromous alewives is likely inaccurate. Like landlocked alewives, adult anadromous alewives can shift zooplankton species

composition and size towards smaller zooplankters (Kircheis et al. 2002), but no studies have identified anadromous alewives as a significant source of larval fish mortality.

YOUNG-OF-YEAR ALEWIFE AND SMALLMOUTH BASS DIET HABITS

Diet overlap between YOY alewives and YOY smallmouth bass was less than is considered biologically important in all lakes analyzed except Meddybemps Lake where the diets of YOY alewives and YOY smallmouth bass were almost identical. There was an almost 90% similarity between stomach contents of the two species in Meddybemps. Ironically, smallmouth bass and alewives have coexisted in Meddybemps Lake since 1877 when bass were introduced by the Maine Commissioners of Fisheries (Warner 2005).

There was much information contained in the genera and species level distinctions of prey items that was not possible to discern in this analysis due to the state of digestion of most diet items. For example, within the family Sididae, *Latona setifera* is a large benthic species, *Sida crystallina* is a littoral species that uses its anal claw to grip submerged vegetation in lake littoral zones, and *Diaphanosoma birgei* tends to be found in deeper areas away from shore (Great Lakes Environmental Research Lab 2004). Sididae size was used as a proxy for species, and indeed YOY smallmouth bass ate significantly larger Sididae than did YOY alewives during the early summer sampling period, but that difference disappeared by the mid summer sampling period. The data indicate that YOY smallmouth bass and YOY alewives were eating similar sized Sididae by August. Like the other lakes sampled for YOY diets, smallmouth bass in Meddybemps had a more diverse diet than YOY alewives, but unlike the other lakes, benthic invertebrates were largely absent from their diet. On average YOY smallmouth bass from Meddybemps Lake were longer than bass from Cathance or Pocomoonshine Lakes but Cathance smallmouth bass had higher condition. An obvious question is why were Meddybemps smallmouth bass not eating benthic invertebrates? Are benthic invertebrates rare in Meddybemps Lake or is intra-specific competition between smallmouth bass high enough that zooplankton are the only available food items? Competition between smallmouth bass and alewives in Meddybemps Lake may be diagnostic of some lake characteristic that limits benthic invertebrate production. Smallmouth bass in Pocumcus also fed heavily on Sididae but YOY smallmouth bass in the other lakes studied consumed benthic invertebrates, which reduced their diet overlap with YOY alewives.

BASS TOURNAMENT RESULTS

Tournament total catch and weight per fish were different between years, but were not different between lakes when grouped based on alewife presence or absence. In particular there was a decline in catch weight in 2005 that was evident in both alewife and no-alewife groups. The pattern was driven by sharp drops in landed weight for Harvey, Magaguadavic and Boyden Lakes. Of these, declines were most dramatic in Harvey Lake; weight per team was approximately half that of the three previous years.

While fishing tournaments and lakes managed for trophy bass can be an economic boon to a region (Chen et al. 2003), catch and release angling can lead to overfishing through initial and delayed mortality and behavioral changes associated with fish relocation that increase nest

and adult mortality. Smallmouth bass are sensitive to hypoxic conditions that might be encountered in livewells, during weigh-in, and after release in some lakes (Furimsky et al. 2003, Edwards et al. 2004). However, estimates of initial and delayed mortality in bass tournaments are generally low, < 5% (Wilde 1998, Edwards et al. 2004), so these factors may not have contributed to the drop in average team weight in 2005. Poor weather conditions on tournament days can negatively affect tournament catches; weather was not considered as an explanatory variable in this analysis. Alternatively, tournament bass released in one area are more likely to be caught again by anglers (Wilde 2003). Although the pattern of lower tournament entries in 2005 is only a strong trend, vigilance would be prudent to ensure the economic viability of smallmouth bass fishing tournaments in Washington County, Maine, and Southwest New Brunswick. Regardless, alewives did not appear to affect the quality of tournament angling.

ALEWIFE GENETIC RELATIONSHIPS

The discovery of landlocked alewives in the St. Croix drainage after 1995 complicated the concern anglers and fishery managers had for alewife effects on smallmouth bass. These concerns included a belief that St. Croix River landlocked alewives may have developed from anadromous stock that were trapped or had assumed a resident life history in the upper reaches of the watershed. However, concerns that the landlocked population in the St. Croix River system developed from, or hybridized with, the anadromous stock are unsubstantiated according to the available genetic data. St. Croix landlocked alewives are of a separate lineage that is reproductively isolated by several generations from anadromous alewives (Bentzen and Paterson 2005).

Anadromous alewives have been stocked as forage fish to supplement other bait fish like *Lepomis* spp. in Maine for landlocked Atlantic salmon, lake trout and age 2+ brown trout (Kircheis and Stanley 1981). Attempts to expand the range of anadromous alewives to interior Maine lakes met with minimal success because stocked adults and their progeny failed to survive past fall of the stocking year (Lackey 1969).

Landlocked alewives also were considered a good forage fish but with better longevity in interior Maine lakes; their small size made them available to most fish predators. One of the first landlocked alewife introductions in Maine were of Cayuga Lake, NY stock, introduced to Echo Lake on Mt. Desert Island, as an alternative to rainbow smelt as the primary forage fish for landlocked salmon and trout (Lackey 1969). Currently there are an estimated 25 lakes in Maine with landlocked alewife populations. Because there were no landlocked populations in New Brunswick in 1995 and present data indicates that the St. Croix River landlocked alewives are not derived from St. Croix River anadromous alewife stock, the landlocked alewives are likely transplants from one of these southern populations.

SUMMARY

The guiding assumption of current smallmouth bass – alewife co-management in the St. Croix drainage has been that anadromous alewives are a negative influence on smallmouth populations and fisheries. This study found no evidence to support this assumption based on historical Maine IFW data, stomach contents data collected in 2005, or tournament fishery data.

Assumptions that anadromous alewives have contributed to landlocked alewife populations through hybridization or by a change in life history also were not supported. Specific results include:

(1) There is no evidence from available historic data in Downeast Maine lakes that the presence of alewives has systematically harmed smallmouth bass in terms of length, condition or growth. The data provide some evidence that bass grew faster in the presence of anadromous alewives than they did in their absence, though these correlational data do not demonstrate a causal relationship.

(2a) Fish constituted only a tiny proportion (less than 0.15%) of the diet of adult anadromous alewives. Alewives were not significant predators on smallmouth bass, although they did feed on other organisms while present in the lakes. This observation was in contrast to literature assertions that anadromous alewives do not feed while spawning.

(2b) In most lakes, young-of-year smallmouth bass and young-of-year alewives did not have an ecologically significant overlap in diet. In the one lake in which diets were similar, populations of bass and alewives have coexisted for over a century. Based on one year's data, therefore, competition for food between the two species did not appear to be important. Given high lake-to-lake and year-to-year variation in ecological conditions, however, additional data would be welcomed.

(3) Smallmouth bass tournament returns in the past few years have been similar in lakes with and lakes without alewives, suggesting that the quality of sport fishing for bass does not differ systematically between lakes with and without anadromous alewives.

(4) Landlocked alewives are genetically distinct from the anadromous alewife populations in the St. Croix and from other studied watersheds. Landlocked and anadromous alewives do not appear to be hybridizing within the St. Croix watershed. Landlocked alewives are almost certainly the result of an independent introduction of landlocked stock from lakes outside the watershed, and not the result of a shift in life history strategy within the watershed.

Based on variability that was seen between lakes and years in this study, the collection and yearly assessment of additional fish and ecosystem data as part of an adaptive management strategy would be prudent. Successful co-management of these species requires, at a minimum (1) *the continued monitoring of the St. Croix anadromous alewife run* and (2) *continued monitoring of individual and population growth rates of smallmouth bass, temperature regimes and food resources in St. Croix lakes with and without anadromous alewives.* Information on (3) *annual fluctuations of prey resources (abundance and distribution of zooplankton, benthic invertebrates and larval fishes)* would provide additional insight into smallmouth bass and alewife interactions.

In addition, this study did not directly address several important factors that may influence smallmouth bass – alewife interactions. These include: (1) *the role of landlocked alewives in the food web of the St. Croix lakes;* future work that focuses on landlocked population size, spatial distribution amongst lakes, demographics, and genetics would be valuable in determining how landlocked alewives might interact with fluctuating anadromous alewife abundance. (2) A*nadromous alewife distribution among watershed lakes;* there is no

information available on how returning anadromous alewives distribute themselves within a series of interconnected lakes, information that would help focus management effort on where the largest impacts in a watershed are likely to occur.

Finally, it is important to reiterate that continued data collection must be accompanied by frequent (yearly, or at least biennial) assessments of the data to allow for adaptive management as conditions change.

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Genetic Analyses of Freshwater and Anadromous Alewife (*Alosa pseudoharengus*) Populations from the St. Croix River, Maine/New Brunswick

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FINAL REPORT

to

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Summary

Through a combination of cloning new microsatellites and screening recently published microsatellite primers from related species, 10 novel microsatellite markers were identified for alewife (*Alosa pseudoharengus*). These 10 microsatellites were used to genotype alewives from four locations in the St. Croix River watershed: anadromous alewives from Dennis Stream and Milltown, and landlocked alewives from East Grand Lake and Grand Falls Flowage. Two other populations of anadromous alewife from the Gaspereau and LaHave Rivers in Nova Scotia were also genotyped, as was one population of blueback herring from the LaHave River. In total, 324 alewives and 30 blueback herring were genotyped.

The 10 microsatellite loci exhibited an average of 10.9 alleles per locus in alewives. Among St. Croix alewives, landlocked populations had less genetic diversity than anadromous populations; for example, the number of alleles per locus and per population averaged 3.64 and 6.91 for landlocked and anadromous alewives, respectively. Levels of genetic diversity in anadromous St. Croix populations were similar to those in anadromous populations from the Gaspereau and LaHave Rivers. This result and other tests suggested that the anadromous alewife populations in the St. Croix have not been subject to a severe genetic bottleneck, although the possibility that there has been some reduction in genetic diversity from historical periods of greater abundance could not be ruled out.

A variety of statistical tests confirmed that anadromous and landlocked populations of alewife in the St. Croix are genetically divergent (F_{ST} = 0.244). These results implied that very little, if any, interbreeding occurs between the two life history types. The results of assignment tests indicated that it should be possible to reliably identify the life history type of individual St. Croix alewives at any life history stage by genotyping them at five microsatellite loci. These results are qualified, however, by the absence of genotypic data from landlocked alewives from the zone of sympatry with anadromous alewives in Woodland Flowage.

Significant genetic differences were observed between anadromous alewife populations in the St. Croix and anadromous populations in the LaHave and Gaspereau Rivers, as well as between the two anadromous St. Croix samples, Dennis Stream and Milltown. These results imply homing of alewives to their natal streams, and consequently, at least partial reproductive isolation between spawning runs, even at the level of tributaries within the St. Croix River. However, the degree of genetic differentiation between the two St. Croix samples was small (F_{ST} = 0.008), and needs to be evaluated further with genetic data from at least one additional year.

Introduction

Two life history types of alewife (*Alosa pseudoharengus*) occur in the St. Croix River system of Maine and New Brunswick: anadromous and landlocked. The abundance of both forms has changed substantially during the last 20 years, but in opposite directions. Anadromous alewives have declined, due in large part to management actions initiated by the State of Maine in 1995 that were aimed at denying access of the anadromous alewives to 98% of their reproductive habitat on the St. Croix, whereas landlocked alewives have increased in abundance in the upper part of the watershed.

This report describes analyses that examine the status of St. Croix alewives from a molecular genetic perspective. The primary goals of this study were to assess the genetic diversity and relationships of St. Croix alewife populations, and to develop methods to differentiate between anadromous and landlocked alewives. Specific objectives were as follows:

1. Development of new genetic markers for the study of alewife populations.
2. Application of the genetic markers to establish methods of reliably identifying anadromous and landlocked alewives in field studies.
3. Application of the genetic markers to determine whether anadromous alewives in the St. Croix have suffered a loss of genetic diversity that could be attributed to the recent population decline.

To facilitate the third objective, the genetic diversity of the St. Croix alewife populations was compared to the genetic diversity of two relatively healthy populations of anadromous alewife in two Nova Scotia rivers, the LaHave and the Gaspereau. Additionally, the alewife populations were compared to a single population sample of blueback herring (*Alosa aestivalis*), in order to ensure that none of the alewife samples were contaminated by mis-identified specimens of this closely related, morphologically similar species.

The molecular genetic markers employed in this study were microsatellites. Microsatellites are DNA sequences composed of di-, tri- or tetranucleotide repeats arrayed in tandem stretches (e.g., GACAGACAGACA…) of tens to hundreds of base pairs. A variety of useful attributes have made microsatellites the most widely employed genetic markers in population studies over the last decade. These attributes and the details of the application of microsatellites to population studies are well described in a number of reviews (e.g. Wright and Bentzen 1994; Jarne and Lagoda 1996; Goldstein and Pollock 1997; Hedrick 1999), but a brief description follows here.

Microsatellites evolve rapidly. The tandem arrays that make up microsatellites mutate at a rate that is several orders of magnitude greater than that of the great majority of (non-repetitive) DNA sequences that make up the genome. The rapid evolution of microsatellites makes them highly polymorphic; that is, each microsatellite exists as a number of allelic variants that differ among individuals and populations.

Most microsatellites are selectively neutral. Unlike genes, they have no particular function or effect on fitness, and hence are largely ‘invisible’ to natural selection. This means that the frequencies of microsatellite alleles vary primarily in response to genetic drift, which causes reproductively isolated populations to diverge in allele frequencies, and to migration

between populations, which causes populations to become more similar. It should be noted that mutation rates in microsatellites, although rapid by evolutionary standards, are not rapid enough to have much influence on population differentiation on contemporary time-scales. Microsatellites gain their chief theoretical advantage as genetic markers because their many allelic variants (produced over much longer time-scales by mutation) make them very sensitive indicators of genetic drift, migration and overall genetic diversity.

The manner in which microsatellites evolve also differs from most DNA sequences. Microsatellite mutations occur primarily in the form of the gain or loss of individual repeating units; therefore, microsatellite arrays evolve by changing length, and length variants of the same microsatellite differ by multiples of the individual repeat element (e.g., di- and tetranucleotide microsatellites differ in increments of two and four base pairs, respectively). By contrast, non-repetitive sequences evolve primarily by base substitutions, with no net change in sequence length. The mode of mutation in microsatellites has a number of implications, but for population studies, the most important one is practical: it facilitates rapid detection and scoring of genetic variation in the microsatellites, because the microsatellite size variation can be rapidly and precisely measured by first selectively amplifying the microsatellite via the polymerase chain reaction (PCR) and then measuring the size of the microsatellite via gel electrophoresis (Figure 1).

The genomes of all eukaryotic organisms contain thousands of microsatellites distributed throughout each chromosome. In order for any particular microsatellite to be utilized as a genetic marker, its sequence, and the sequence of the non-repetitive DNA immediately flanking it, need to be known. Given this information, DNA primers can be designed that allow the microsatellite to selectively amplified, and its genotype determined. At the outset of this study, there were no published DNA sequences for alewife microsatellites; hence, a critical initial research objective was to develop microsatellite markers for alewife. We took two approaches to meet this objective. The first approach was to clone and sequence microsatellites directly from alewife DNA. The second approach relied on the fact that microsatellites and their flanking sequences are often relatively well conserved in closely related species. This means that primers designed for one species can often be used to amplify microsatellites in congeneric species. Therefore, we also tested 14 primer sets recently published for other species of *Alosa* (Waters et al. 2000; Faria et al. 2004) to determine if any of them could be used on alewife. This combined approach allowed us to identify 10 microsatellite loci that could be reliably amplified and genotyped in alewife.

Methods

Samples

Anadromous alewives were collected during their spawning migration at Milltown Dam fishway (river kilometre 2.26), St. Croix River, June 7-9 2004, and at Cranberry Lake fishway on Dennis Stream, a tributary of the St. Croix River, May 24 2004, by D. McLean and L. Sochasky. Landlocked alewives were collected at Grand Falls dam turbine intake, Oct. 28 and Nov. 11-13 2003, by J. Dow and L. Sochasky, and at East Grand Lake, June 24-July2 2004, by M. Smith. Anadromous alewives from the Gaspereau River, Nova Scotia, were collected during their spawning migration, May 21-22 2000, at the fish ladder bypassing the White Rock Generating Station by J. Gibson. Anadromous alewives and blueback herring were collected from the

LaHave River, Nova Scotia, May 2003, by Canadian Department of Fisheries and Oceans (DFO) personnel.

Microsatellite marker development for alewife

Novel microsatellites were isolated using a magnetic bead hybridisation technique. Genomic DNA was purified from fin tissue of a LaHave River alewife. This DNA was used to create microsatellite enriched libraries for CATC, and GACA repeats, following previously published protocols (Hamilton *et al*. 1999; Diniz *et al*. 2004). The microsatellite libraries were cloned using Qiagen cloning vector, transformed into Max Efficiency DH5α (Invitrogen) competent cells, and plated on imMedia AmpBlue agar (Invitrogen). Cloning yielded 224 insert-bearing colonies that were screened for suitably sized inserts (400-1000bp) by direct polymerase chain reaction (PCR) amplification of colony picks using M13 primers under standard PCR conditions, and imaged with agarose electrophoresis. Next, 114 PCR products were cleaned on size exclusion filters (Omega10K molecular weight cut-off filters, Pall) and sequenced on a CEQ 8000 genetic analyser (Beckman Coulter). Of the 114 sequenced DNA clones, 12 were judged suitable for further development. Forward and reverse primers were designed for these sequences using PRIMER3 software (Rozen & Skaletsky 2000). The 12 novel primer sets, as well as six primer sets originally designed for *Alosa sapidissima* (Waters et al. 2000) and eight primer sets designed for *Alosa fallax* and *Alosa alosa* (Faria et al. 2004) were tested on a panel of 4-16 alewife DNA samples.

DNA isolation and genotyping

Genomic DNA was extracted from fin clips stored in ethanol (St Croix, Lahave) or scales stored in scale envelopes (Gaspereau River). Either <10 mg of fin tissue or 2 scales per fish were incubated overnight at 55°C and shaken at 250 rpm in 150 µL digestion buffer (100 mM NaCl, 50 mM Tris·HCl pH 8, 10 mM EDTA, 0.5% SDS, 40 µg Proteinase K). DNA isolation was performed using a glassmilk-binding protocol following Elphinstone *et al*. (2003) and modified for automation in 96 well format using a Perkin Elmer MPII liquid handling robot.

Genetic data were collected for 10 microsatellite loci using primers listed in Table 1. Amplifications were conducted in 5 µL or 10 µL reaction volumes containing 20 mM Tris-HCl pH8.8, 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 2 mM $MgSO_4$, 0.1% Triton X-100, 200 uM dNTPs, 100-200 nM fluorescently labelled primer, 100-200 nM unlabelled primer, 0.25-0.5 U *Taq* DNA polymerase, and ~50-100 ng template DNA. Amplifications were performed in MJ Research or Eppendorf thermocyclers using an initial 94°C 3 min denaturing step, then 30 cycles of 94°C 30s, primer specific T_A (see Table 1) 30s, 72°C 30s, and a final 72°C 2m step. PCR products were visualized in 6% denaturing PAGE gels on either LiCor IR2 DNA analyzers (locus Asa8) or on an FMBioII (all other loci). Microsatellite alleles were sized on both instruments by reference to DNA standards composed of known microsatellite alleles run on each gel. Two measures were taken to ensure consistency and reproducibility of genotyping across all populations and loci. Two fish from each population were routinely genotyped twice at each microsatellite locus, and a 'standard' fish from one population (LaHave) was included when all other populations were genotyped at each microsatellite. A minimum of 50 fish were

successfully genotyped at each locus for each population (except blueback herring, where the sample size was 30).

Statistical analyses

The number of alleles per locus, observed heterozygosity (H_O), unbiased estimates of heterozygosity (H_E, Nei 1987) within populations and estimates of allelic richness (Ae, a measure of allelic diversity standardized to a common sample size) were calculated using FSTAT (v. 2.9.3.2; Goudet 2002). Tests of departures from Hardy-Weinberg equilibrium (HWE), genotypic linkage disequilibrium, and log likelihood (G)-based tests of genic differences among populations were performed using GENEPOP (v. 3.4; Raymond & Rousset 1995). Tests for departures from HWE were performed for each locus-population combination using an exact test where the *P*-values were estimated without bias using a Markov chain method following the algorithm of Guo & Thompson (1992). Tests for genotypic linkage disequilibrium for all pairs of loci within each population and tests for allelic heterogeneity between populations were also made using the Markov chain method. Log-likelihood (G)-based exact tests were performed at each locus and over all loci among all populations, and between all possible population pairs by estimating an unbiased *P*-value. For all Markov chain tests, default parameters in GENEPOP for dememorization number, batches and iterations were invoked. Tests were combined across loci or populations using Fisher's method. F_{ST} was calculated following Weir and Cockerman (1984), and the significance of F_{ST} was evaluated using a permutation test implemented in GENETIX (v. 4.05, Belkhir 1996-2004). Sequential Bonferroni adjustments were used to judge significant levels for all simultaneous tests in this study (Rice 1989) with an initial α level of 0.05.

The program STRUCTURE (v. 2.1; Pritchard *et al.* 2000) was used to group genotypes of St. Croix alewives into clusters of individuals that minimized departures from Hardy-Weinberg and linkage equilibrium. Three iterations were run for each value of *k* (the putative number of clusters) using the admixture model, with burn-in and Monte Carlo Markov chain values of 100,000 each, and values of $k = 1$ through 8. For each value of *k*, the program estimated the proportion of each individual's genotype that was derived from each of the *k* putative clusters (populations).

The success with which particular populations could be either identified or excluded as the population of origin of individual alewives within the St. Croix River system was estimated using procedures implemented in the program GENECLASS2 (Piry et al. 2004). For each alewife, the likelihood of its genotype occurring within its source population, or within other populations under consideration was calculated using the method of Paetkau et al. (1995). This method assumes that each potential source population is a group of randomly mating individuals, with allele frequencies estimated from the sample of individuals drawn from that population. When the likelihood of an individual's genotype is being estimated for the population from which it was sampled, that individual's genotype is removed from the calculation of allele frequencies for that population (the leave-one-out procedure). Next, the probability of occurrence of a particular genotype within a given population is estimated by comparing the likelihood of its genotype relative to the genotype likelihoods of 10,000 simulated individuals for that population, where the simulated individuals are created according to the resampling method of Paetkau et al. (2004). Not all loci are equally useful for identifying the population of origin of individuals. Shriver et al. (1997) showed that the relative informativeness of genetic loci is predicted by δ, where δ is defined as the sum of allele frequency differences of like sign for a particular locus

between two populations. In order to obtain the most efficient discrimination of anadromous and landlocked alewives, δ values were calculated for all loci for comparisons of pooled samples of anadromous and landlocked St. Croix alewives, and the five loci with the highest values were used in the assignment analysis.

The program BOTTLENECK (Piry et al. 1999) was used to test for evidence of a population bottleneck in each of the four St. Croix alewife population samples. Two tests conducted by bottleneck were applicable to the alewife data. The first test, a Wilcoxon sign-rank test, tested for evidence of excess heterozygosity relative to drift-mutation equilibrium expectations under three different mutational models that would be expected following a bottleneck. The second test examined the allele frequency distributions of individual loci to determine whether there had been a mode shift from the characteristic "L shaped" distribution expected under drift-mutation equilibrium.

A neighbor-joining (NJ) tree based on Cavalli-Sforza Edwards genetic distances between alewife populations was created using the program NJBP (Jean-Marie Cornuet, INRA Laboratoire de Modélization et Biologie Evolutive, Montpellier). Statistical support for each cluster of populations on the NJ tree was estimated by bootstrapping across loci (2000 replicates) using NJBP. Multidimensional scaling plots based on estimates of F_{ST} between pairs of populations were generated using the software package Primer V 5.2.4 (2002), and based on 100 iterations.

Results and Discussion

Microsatellite marker development

Of the 12 novel primer sets designed for alewife, three (Aps1, Aps2A, Aps7) yielded high quality amplifications of polymorphic microsatellite loci in a test panel of 16 alewives and were retained for further study. The other nine primer sets either produced PCR products of unreliable quality (four loci), amplified non-polymorphic microsatellites (four loci), or failed to amplify at all (one primer set). Screening of 14 other primer sets developed for three other species of *Alosa* yielded an additional seven primer sets that produced high quality amplification of polymorphic microsatellites in alewife: Asa8 (Waters et al. 2000), and Aa14, Aa15, Aa16, Af6, Af11, Af13 and Af20 (Faria et a. 2004). We subsequently genotyped an average of 322 alewives at each of these 10 microsatellite loci, and an average of 27 blueback herring at nine loci (Asa8 was only amplified successfully in 11 individuals of in this species).

Data quality: tests of linkage disequilibrium and departure from Hardy Weinberg equilibrium

Most multilocus analyses of genetic data assume that the loci used are in linkage equilibrium; that is, allelic states at one locus are independent of those at other loci. We tested for linkage disequilibrium between all pairs of loci in the six alewife and single blueback population and obtained 13 significant results ($P \leq 0.05$), fewer than would be expected by chance in the 315 pairwise comparisons. For this reason, and because the linkage disequilibrium tended to involve different pairs of loci in different populations, we concluded that there was no basis to exclude loci from subsequent analyses on the basis of linkage.

Multilocus analyses also usually assume that the loci are in Hardy Weinberg equilibrium; that is, the frequencies of different single locus genotypes are consistent with expectations from random mating within populations. The results of tests for departures from HWE in the alewife and blueback data sets were generally non-significant, with a couple of exceptions (Table 2). The locus Aps7 showed a significant deficit of heterozygotes (F_{IS} [a measure of departure from HWE] = 0.235; P = 0.0057) in the Milltown sample. A combined test suggested that Aps7 also departed significantly from HWE across populations (P = 0.0166); however, this result was not significant following Bonferroni correction for 10 simultaneous tests. A combined test also suggested a net departure from HWE across loci for the Milltown sample; again however, this was not significant following correction for multiple tests (P = 0.0141; 6 tests). The blueback herring sample exhibited a significant deficit of heterozygotes at locus Af20 (F_{IS} = 0.231, P = 0.0038). Tests of departure from HWE are primarily useful for two reasons: (1) to identify loci subject to null alleles or genotyping errors that might distort subsequent analyses; (2) to identify samples that might be an admixture of multiple randomly mating populations. Null alleles and genotyping errors tend to be manifested in multiple significant test results of the same locus across populations, whereas population admixtures are expected to be apparent in departures from HWE across multiple loci within a single population sample. The HWE test results provided little evidence of either sort of problem for the current data set; hence all loci and population samples were retained for further analyses.

Genetic diversity across loci and populations

Genetic diversity varied substantially across loci and populations (Table 2). The number of alleles per microsatellite locus varied from three (Aps1) to 32 (Af20), with a mean of 10.9 alleles per locus in alewives. Among St. Croix River system populations, genetic diversity was markedly higher in the two anadromous samples, Dennis Stream and Milltown, than it was in the two landlocked samples, Grand Falls Flowage and East Grand Lake (Figure 2). For the two anadromous samples, the mean number of alleles per locus, mean allelic richness (a measure of the number of alleles for a standard sample size across all populations) and mean unbiased expected heterozygosity were 6.91, 6.54 and 0.54, whereas for the landlocked samples, the corresponding values were 3.64, 3.48 and 0.30. Genetic diversity values for the LaHave and Gaspereau River alewives, and for the LaHave River blueback herring, were similar to those for the anadromous alewives in the St. Croix River system, and are discussed in greater detail below.

***How many populations are present in the St. Croix?* STRUCTURE analysis**

We used the program STRUCTURE to estimate the number of populations present in the St. Croix samples independently of information about sampling site and ecotype (anadromous vs. landlocked). Simulation results obtained with STRUCTURE revealed that the most probable value for k (the number of populations) was 2, although values of k of 3-8 were all more probable than k = 1 (Table 3). These results thus support the presence of at least two genetically distinct populations of alewife in the St. Croix system.

STRUCTURE allows the proportion of each individual's genotype that is derived from a particular population cluster to be estimated. Individuals whose genotypes are entirely or mostly characteristic of a single cluster are assumed to derive most or all of their recent ancestry from that cluster. Conversely, individuals whose genotype is an admixture of different clusters may be of mixed recent ancestry. Estimates of the genetic admixture proportions for each individual St.

Croix alewife assuming the presence of $k = 2$ populations revealed a strong distinction between anadromous and landlocked alewives (Figure 3). All landlocked alewives were estimated to derive all or nearly all of their genotypes from a single cluster, and all anadromous alewives were estimated to derive all or most of their genotypes from a second cluster. Thus, there was little evidence for admixture (hybridization) between the two groups of alewives, although the genotypes of a few alewives (most notably three fish from Dennis Stream) were consistent with the possibility of some mixed ancestry (Figure 3).

The STRUCTURE results demonstrate the presence of two genetically distinct groups of alewife in the St. Croix River drainage that correspond perfectly to their previously identified life history type. The STRUCTURE results do not, however, prove that there are only two populations of alewife represented by the fish examined here. The strengths of the STRUCTURE analysis are that no prior assumptions are made about the population affinities of individual fish, and also that the admixture proportions of each fish's genotype can be estimated. However, analyses that do not involve prior classification of individuals into groups may lack the power to detect subtle genetic differentiation that can occur between closely related (but nonetheless demographically distinct) populations.

How many populations are present in the St. Croix? Tests of heterogeneity and genetic differentiation

Genic tests of allele frequency heterogeneity revealed significant differences between each pair of population samples from the St. Croix system ($P \leq 0.0034$; Table 4). Likewise, values of F_{ST} (a measure of genetic differentiation) were significantly greater than zero for all pairs of populations within the St. Croix system (Table 5). The magnitude of genetic differentiation was an order of magnitude greater between anadromous and landlocked populations (mean $F_{ST} = 0.244$) than between populations within life history type (Dennis vs. Milltown $F_{ST} = 0.008$; Grand Falls vs. East Grand $F_{ST} = 0.023$).

Can alewives be reliably identified to population on the basis of their microsatellite genotypes? Assignment tests

The STRUCTURE results demonstrated that it should be possible to determine whether individual alewives are anadromous or landlocked, on the basis of their microsatellite genotypes. Moreover, it should also be possible to identify hybrids between the two ecotypes with relative certainty. As a further test of our ability to identify the life history type of individual alewives, we conducted assignment tests on St. Croix alewives using the program GENECLASS2. For these tests, we pooled the alewives according to life history type, since we were primarily interested in distinguishing the alewives at this level, and not at the level of individual populations within life history type. We calculated the δ values for the 10 microsatellite loci to determine which loci would be most useful for identifying the population of origin (life history type). Five loci (Af20, Af11, Aps7, Af13 and Af6) had δ values greater than 0.45; we used these loci to test the ability of GENECLASS2 to correctly identify the life history type of St. Croix alewives.

GENECLASS2 assigned 100% of St. Croix alewives correctly to life history type; that is, their multilocus genotypes were estimated to be more probable in the population from which they were sampled, than in the alternate population. Since we are also interested in the power of the assignment procedure to reject the incorrect population as a potential source for a fish, we

examined the probability values for the genotypes of individual alewives in the 'wrong' population. This analysis revealed that a landlocked origin could be rejected for 99% of anadromous alewives with at least 99% probability. On the other hand, an anadromous origin could be rejected for only 56% of landlocked alewives with 99% certainty; although an anadromous origin could be rejected for 97% of landlocked alewives with at least 90% probability (Figure 4). The somewhat weaker ability of the assignment procedure to rule out anadromous origins for landlocked alewives presumably results from the lesser genetic variability of the landlocked alewives. Because the genetic variation in landlocked alewives is mostly a subset of the substantially greater genetic variation present in anadromous alewives (Figure 2), the genotypes of some landlocked alewives can potentially occur in anadromous populations (although usually with low probability), whereas the great majority of anadromous genotypes can be effectively excluded from originating in the landlocked population because they have alleles that are rare or non-existent in the landlocked population. Notwithstanding this asymmetry in the assignment test results, the important point remains that the life history type of the great majority of St. Croix alewives can be correctly identified with strong statistical certainty.

Have the anadromous alewives in the St. Croix River experienced a recent genetic bottleneck?

The amount of genetic variation in populations represents a dynamic balance between several evolutionary forces. Genetic drift within populations leads to the loss of genetic variation. This loss of genetic variation is offset by 'new' genetic variation that comes either from new mutations, or from migrants that bring genetic variants from other populations. For anadromous populations of fish such as alewives, immigration from neighboring populations is expected to replenish genetic variation much more rapidly than mutation. The rate at which genetic drift occurs is inversely proportional to population size; hence a population that experiences a decrease in size is expected to experience rapid genetic drift, and an accelerated loss of genetic variation. The amount of genetic variation that is lost (and hence the severity of the genetic bottleneck) is correlated with the severity and the duration of the demographic bottleneck. The most severe genetic bottlenecks occur when populations are at low levels for many generations; whereas, short-term population declines followed by recovery may entail the loss of relatively little genetic variation, particularly in a species with overlapping generations, and relatively high rates of migration (straying) among populations.

We looked for evidence of a genetic bottleneck in anadromous St. Croix alewives by comparing levels of genetic variation in the Dennis Stream and Milltown samples to levels of genetic variation in the two other anadromous populations, LaHave River and Gaspereau River. These comparisons yielded no strong indication that anadromous alewife populations in the St. Croix have suffered a loss of diversity. Three measures of genetic diversity for anadromous St. Croix populations (all averaged over loci and between the two populations), the number of alleles (A), allelic richness (Ae) and expected heterozygosity (He) were all intermediate to values for the two other populations (Table 2; St. Croix A = 6.91, Ae = 6.54, He = 0.54; Gaspereau A = 5.82, Ae = 5.55, He = 0.47; LaHave A = 8.18, Ae = 7.42, He = 0.57). These results do not, however, rule out the possibility that the St. Croix populations have suffered some loss of genetic diversity as a result of a recent population decline. Under equilibrium conditions, genetic diversity is expected to be positively correlated with population size, and since the St. Croix River has the largest watershed of the three rivers, historically it may also have had the largest

alewife population, and therefore may originally have had greater genetic diversity than either the LaHave River or Gaspereau River populations.

The possibility that there may have been some decline in genetic diversity in anadromous St. Croix River alewives is suggested by the lower A and Ae values for the main stem Milltown sample compared to Dennis Stream (6.64 and 6.30 vs. 7.18 and 6.78). Normally, one would expect the (presumably) historically larger main stem population to exhibit more genetic variation than the tributary population, unless mixing between the two populations is great enough to homogenize genetic variation between the two populations. Thus, the slightly lower genetic diversity in the main stem population sample could reflect a loss of genetic diversity in this population. Note that expected heterozygosity did not follow the trend of the other two indicators (He = 0.56 vs. 0.51 for Milltown and Dennis); however, allelic diversity normally declines more rapidly than heterozygosity following a bottleneck.

To further investigate the possibility that any of the St. Croix alewife populations have suffered a genetic bottleneck, we applied two additional tests for evidence of genetic bottlenecks, both implemented in the program BOTTLENECK (Piry et al. 1999). The first test, a Wilcoxon sign-rank test, did not detect significant excess heterozygosity relative to mutation-drift expectations (under any of three different mutational models that could apply to microsatellites) in any of the four St. Croix alewife population samples ($P > 0.05$). Likewise, BOTTLENECK determined that the allele frequency distributions of microsatellite loci in all four St. Croix alewife populations approximated the L-shaped distribution expected under drift-mutation equilibrium. Again, these results do not conclusively rule out the possibility of a loss of genetic diversity in the St. Croix populations, but they do suggest that there has been no severe bottleneck. Interestingly, the BOTTLENECK results also suggested that the substantially lower genetic diversity in the landlocked populations is itself not the result of a very recent bottleneck, such as might have occurred in the last few generations.

Relationships among alewife populations, and between alewife and blueback

A dendrogram based on Cavalli-Sforza Edwards genetic distances among populations supported the clear genetic distinction between landlocked and anadromous alewives (Figure 5). The tree revealed two clusters distinguished by 100% statistical support, one comprised of the two landlocked samples, and the other containing all of the anadromous populations. Dennis Stream and Milltown also clustered together on the tree, as expected for two populations from the same river system; however, the statistical support for this grouping was modest, 74%.

We also examined the relationships of the various populations via multidimensional scaling (MDS) based on F_{ST} values between populations (Table 5). Although not as amenable as dendrograms to statistical tests of population groupings, MDS better captures the multidimensional nature of genetic distances (including F_{ST}) among a group of populations. MDS revealed three distinct groups of populations when blueback was included in the analysis (Figure 6a): blueback, anadromous alewife, and landlocked alewife. When blueback was excluded from the analysis, the remaining two groups were still evident, however the divergence among alewife populations was more apparent. As in the dendrogram, however, the key pattern that emerged was the strong distinction between the landlocked and anadromous alewife populations, and the relative similarity of all anadromous populations (Figure 6b).

Conclusions, caveats and suggestions for further research

Genetic diversity was lower in landlocked alewives than in anadromous alewives. Both landlocked alewife populations had fewer alleles and lower heterozygosity than anadromous populations in the St. Croix River or elsewhere, signaling a lower longer term effective population size (Ne) in the landlocked populations than in any of the anadromous populations. In contrast, there was no strong evidence of a genetic bottleneck in either of the anadromous St. Croix, although slightly lower genetic diversity in the Milltown sample than in the Dennis Stream sample suggests the possibility that there has been some loss of genetic variation in the main stem population.

Each population sample differed significantly from all others in terms of allele frequencies and F_{ST}. For anadromous and landlocked St. Croix alewife populations, genetic differentiation was very strong, implying that these populations undergo very little, if any interbreeding. One practical consequence is that it should be straightforward to reliably identify the life history type of alewives of any life history stage, using microsatellite markers identified in this study. One caveat is that this study did not examine any landlocked alewives from Woodland Flowage, a zone of sympatry between landlocked and anadromous alewives. It would clearly be desirable to genotype samples of landlocked alewife from Woodland Flowage to determine whether they show any evidence of genetic admixture with anadromous alewife.

Although statistically significant, the genetic divergences among anadromous alewife populations were substantially less than those between the landlocked and anadromous populations. Such F_{ST} values (0.01-0.04) are consistent with homing of alewives to their natal rivers, but with some straying. This implication is unsurprising for alewife spawning in rivers separated by hundreds of kilometers of ocean; although, to our knowledge, such homing has not previously been demonstrated by genetic markers for alewife. More surprising is the small but significant genetic difference between the Dennis Stream and Milltown samples ($F_{ST} = 0.008$). This result implies significant homing on the geographically fine scale of tributaries within a river system. This conclusion should definitely be regarded as tentative. Genetic analysis of Dennis Stream and Milltown alewives from at least one additional year is needed to determine whether or not the relatively subtle genetic differentiation between the two river branches is stable.

Our chief recommendations for further work in the short term, therefore, are to genetically analyze additional alewife samples: principally anadromous St. Croix alewives from at least one additional spawning season from both Dennis Stream and the main stem of the river, and landlocked alewives from Woodland flowage if these become available. In addition, it would be helpful to compare St. Croix alewives to other anadromous populations in more geographically proximate rivers than the Gaspereau and LaHave Rivers, in order to investigate the relatedness of St. Croix alewife populations to those in nearby rivers. It would be useful to explore our ability to genotype alewives recovered from the gut contents of predatory fishes, in order to determine if we can identify the population origins of these prey items. Finally, if scale samples of anadromous alewives predating 1995 should be available, it would be extremely useful to genotype these scales, because in this way we could directly assess whether there has been any loss of genetic diversity in the main stem alewife population of the St. Croix River.

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Table1. Primers used to amplify microsatellites in alewife and blueback herring.

Locus	Repeat motifs	Primer sequence (5'-3')	T_A (°C)	Size range (bp)	Reference
Aa14	$(GT)_8$	GAGAAGAGGGCATTCG ATTTAGTGTGTGCCCAGC	60	116-192	Faria *et al.* 2004
Aa16	$(CA)_4aa(CA)_3aa(CA)_8$	TTGACCGAGCGCAAACTG TGACACTGACTCATCATGC	58	115-139	Faria *et al.* 2004
Af11	$(CA)_5ct(CA)_4$	CGAGTACAATCAAAAGCC AGCTTCCTCAGACTGG	52	123-145	Faria *et al.* 2004
Af13	$(CA)_{17}$	AGGATACATAGTCTCCC CAAGTTGGAGTGTCACG	57	156-188	Faria *et al.* 2004
Af20	$(CA)_{11}$	AATGGACATATCTGCTGG ATGGAGGGCCATATTTCG	58	170-336	Faria *et al.* 2004
Af6	$(CA)_4at(CT)_5(CA)_{16}aa(CA)_8$	AGGAGATGTTTATCCTGCC CACAGAGGCATAAATGTGG	58	160-194	Faria *et al.* 2004
Aps-MGPL-1	$(CTGT)_8$	CTGCACGTCTGACTGTCTGC TATGGGATGGATGGGATCAG	62	84-112	This study
Aps-MGPL-2A	$(TCAA)_8$	CCAGTTACGTCAGTCACACGA TGGGCAGACAACAGAAGTTTT	60	77-133	This study
Aps-MGPL-7	$(CATC)_{14}$	TCCTCTCTCACCACAGGTCTC ATAGGCTGAATCAGGGAAGG	63	97-133	This study
Asa-8	$(TTTG)_8$	TCCATTCCATTACGTAGAGCACT CCGGCAGGGCACAGAAC	58	113-129	Waters *et al.* 2000

Table 2. Summary statistics for microsatellite data in alewife and blueback populations.

LOCUS		Dennis	Milltown	Grand Falls	East Grand	Gaspereau	LaHave	*Avg/*total	blueback
Aa14	N	52	51	51	51	56	61	*53.7*	30
	A	18	19	7	9	13	22	29	15
	Ae	16	16.8	6.9	8.1	12.2	19.2	*16.1*	--
	Ac	116	116	116	116	116	116	--	146/148
	Af	0.53	0.47	0.46	0.38	0.58	0.39	*0.468333*	0.2
	R	66	76	54	58	66	70	--	44
	He	0.705	0.753	0.711	0.723	0.648	0.827	*0.728*	0.879
	Ho	0.731	0.753	0.765	0.706	0.679	0.803	*0.739*	0.767
	Fis	-0.037	-0.095	-0.076	0.023	-0.047	0.029	*-0.034*	0.144
	P	0.2445	0.9384	0.9735	0.9367	0.7378	0.1227	0.9242	0.4548
Af13	N	52	51	50	52	56	61	*53.66667*	24
	A	9	9	6	7	8	11	14	5
	Ae	8.6	8.3	6	6.8	7.6	9.6	*9.3*	--
	Ac	172	174	182	184	182	172	--	164
	Af	0.269	0.275	0.28	0.567	0.375	0.238	*0.334*	0.397
	R	24	24	16	16	24	32	--	32
	He	0.804	0.776	0.794	0.642	0.782	0.807	*0.767*	0.547
	Ho	0.827	0.706	0.740	0.615	0.821	0.787	*0.749*	0.621
	Fis	-0.029	0.091	0.068	0.042	-0.052	0.025	*0.024167*	-0.138
	P	0.8012	0.0024	0.7583	0.2691	0.399	0.3645	0.0785	0.0276
Asa8	N	51	51	52	52	54	61	*53.5*	11
	A	5	5	2	1	5	5	5	3
	Ae	5	5	1.8	1	4.7	5	*4.9*	--
	Ac	125	125	125	125	125	125	--	125
	Af	0.608	0.618	0.99	1	0.704	0.689	*0.768*	0.909
	R	16	16	4	0	16	16		16
	He	0.576	0.576	0.019	0	0.479	0.492	*0.357*	0.177
	Ho	0.628	0.608	0.019	0	0.482	0.459	*0.366*	0.1818
	Fis	-0.091	-0.055	0	-	-0.005	0.067	*-0.017*	-0.026
	P	0.8952	0.6162	-	-	0.4235	0.3736	0.7809	1
Aps2a	N	52	51	52	52	56	61	*54*	30
	A	6	7	4	3	5	8	12	7
	Ae	5.7	6.6	3.7	2.9	4.7	7.3	*7.6*	--
	Ac	77	77	77	77	77	77	--	85
	Af	0.529	0.608	0.885	0.894	0.625	0.492	*0.672*	0.55
	R	20	56	48	44	20	52	--	30
	He	0.636	0.587	0.212	0.194	0.563	0.701	*0.482*	0.650
	Ho	0.577	0.628	0.212	0.173	0.589	0.721	*0.483*	0.733
	Fis	0.094	-0.07	0	0.11	-0.047	-0.029	*0.009667*	-0.13
	P	0.0863	0.1063	0.2963	0.4454	0.1593	0.1503	0.0488	0.6446

Table 2 continued

LOCUS		Dennis	Milltown	Grand Falls	East Grand	Gaspereau	LaHave	*Avg/*total	blueback
Af20	N	52	52	52	52	56	61	*54.16667*	30
	A	20	12	12	8	15	17	32	10
	Ae	18.6	11.7	10.7	7.8	14.2	15	*17.8*	--
	Ac	228	228	212	212	208	236	--	182
	Af	0.183	0.353	0.731	0.635	0.277	0.328	*0.417833*	0.533
	R	38	34	56	38	54	74		60
	He	0.918	0.818	0.457	0.563	0.867	0.846	*0.745*	0.691
	Ho	0.846	0.843	0.462	0.462	0.964	0.820	*0.733*	0.533
	Fis	0.079	-0.031	-0.009	0.182	-0.113	0.031	*0.023*	0.231
	P	0.4744	0.212	0.3927	0.0121	0.3466	0.7858	0.1008	0.0038
Aps7	N	51	52	52	52	56	61	*54*	30
	A	7	7	4	4	5	8	8	3
	Ae	6.9	6.9	4	3.9	4.7	7.8	*6.7*	--
	Ac	113	113	125	125	113	109	--	97
	Af	0.327	0.382	0.779	0.885	0.509	0.262	*0.524*	0.9
	R	24	24	20	20	24	28		16
	He	0.769	0.742	0.380	0.213	0.635	0.789	*0.588*	0.186
	Ho	0.75	0.569	0.365	0.231	0.536	0.623	*0.512*	0.2
	Fis	0.025	0.235	0.038	-0.085	0.157	0.212	*0.097*	-0.077
	P	0.7999	0.0057	0.5662	1	0.0578	0.0256	0.0166	1
Aps1	N	52	51	50	52	56	60	*53.5*	30
	A	2	2	1	1	2	3	3	3
	Ae	2	2	1	1	2	3	*2.3*	--
	Ac	84	84	84	84	84	84	--	104
	Af	0.952	0.971	1	1	0.946	0.917	*0.964*	0.883
	R	4	4	0	0	4	10	--	28
	He	0.092	0.058	0	0	0.102	0.157	*0.068*	0.216
	Ho	0.096	0.059	0	0	0.107	0.133	*0.066*	0.167
	Fis	-0.041	-0.02	-	-	-0.048	0.154	*0.01125*	0.233
	P	1	1	-	-	1	0.0805	0.7535	0.0723
Aa16	N	52	51	51	52	56	61	*53.83333*	30
	A	3	3	1	1	3	5	5	7
	Ae	2.8	3	1	1	3	4.3	*3.1*	--
	Ac	137	137	137	137	137	137	--	135
	Af	0.914	0.775	1	1	0.902	0.787	*0.896333*	0.533
	R	24	24	0	0	24	24	--	24
	He	0.161	0.368	0	0	0.183	0.333	*0.174*	0.670
	Ho	0.135	0.353	0	0	0.161	0.344	*0.165*	0.633
	Fis	0.166	0.04	-	-	0.124	-0.039	*0.07275*	0.055
	P	0.3144	0.7346	-	-	0.1094	1	0.4987	0.0632

Table 2 continued

LOCUS		Dennis	Milltown	Grand Falls	East Grand	Gaspereau	LaHave	*Avg/*total	blueback
Af6	N	52	51	50	52	52	60	*52.83333*	26
	A	5	5	3	3	4	7	8	14
	Ae	5	5	3	3	4	6.4	*6.1*	--
	Ac	166	166	166	166	166	166	--	190
	Af	0.442	0.4	0.57	0.558	0.731	0.533	*0.539*	0.212
	R	26	26	4	4	24	26	--	34
	He	0.596	0.727	0.546	0.560	0.443	0.633	*0.584*	0.882
	Ho	0.385	0.72	0.56	0.615	0.385	0.567	*0.5386*	1
	Fis	0.111	0.009	-0.026	-0.099	0.133	0.106	*0.039*	-0.137
	P	0.5964	0.8576	1	0.6545	0.2882	0.3347	0.87	0.6596
Af11	N	52	50	51	51	56	61	*53.5*	26
	A	4	4	1	2	4	4	4	3
	Ae	4	4	1	2	4	4	*4*	--
	Ac	139	139	145	145	139	139	--	145
	Af	0.385	0.539	1	0.975	0.723	0.385	*0.668*	0.942
	R	22	22	0	6	22	22	--	22
	He	0.385	0.727	0	0.560	0.443	0.714	*0.472*	0.112
	Ho	0.596	0.72	0	0.615	0.387	0.754	*0.512*	0.115
	Fis	-0.08	0.135	-	-0.013	0.086	-0.056	*0.014*	-0.027
	P	0.4978	0.1144	-	1	0.5247	0.2546	0.4621	1
Average A		7.2	6.6	3.7	3.5	5.8	8.2		6.4
Average Ae		6.8	6.3	3.6	3.4	5.6	7.4		
Average He		0.513	0.557	0.284	0.314	0.468	0.576		0.455
PHWE combined		0.773	0.0141	0.9137	0.4457	0.2484	0.0743		0.0394

N, number of fish genotyped; A, number of alleles; Ae, allelic richness; Ac, most common allele; Af, frequency of most common allele; R, range of allele sizes (in bases); He, unbiased expected heterozygosity; Ho, observed heterozygosity; Fis, inbreeding coefficient for individuals; P, probability of departure from Hardy Weinberg equilibrium (2-tailed, and based on Fisher's combined test for comparisons across populations or loci).

Table 3. Ln(P) values for different values of *k* in STRUCTURE analyses.

	Ln (P)				
k	**Run 1**	**Run 2**	**Run 3**	**Mean**	**SD**
1	-4997	-5002.1	-5014.2	-5004.4	8.83
2	-4145.7	-4145.7	-4146.5	-4146.0	0.46
3	-4194.4	-4201.9	-4227.7	-4208.0	17.47
4	-4240.8	-4289.6	-4318.8	-4283.1	39.41
5	-4301	-4378.4	-4442.9	-4374.1	71.05
6	-4310.5	-4386.8	-4500.2	-4399.2	95.45
7	-4468.4	-4559.3	-4274.2	-4434.0	145.64
8	-4484	-4480.5	-4585.6	-4516.7	59.70

Table 4. Probability values for genic tests of homogeneity between pairs of populations.

POPULATION	**Dennis**	**Milltown**	**Grand Falls**	**East Grand**	**Gaspereau**	**LaHave**
Dennis	--	0.00112	< 0.00001	< 0.00001	< 0.00001	< 0.00001
Milltown		--	< 0.00001	< 0.00001	< 0.00001	< 0.00001
Grand Falls			--	0.00337	< 0.00001	< 0.00001
East Grand				--	< 0.00001	< 0.00001
Gaspereau					--	< 0.00001
LaHave						--

Table 5. F_{ST} values for pairs of populations (above diagonal) and associated probability values (below diagonal).

POPULATION	**Dennis**	**Milltown**	**Grand Falls**	**East Grand**	**Gaspereau**	**LaHave**	**blueback**
Dennis	--	0.008	0.232	0.251	0.042	0.014	0.370
Milltown	0.004	--	0.239	0.254	0.044	0.023	0.372
Grand Falls	<0.0001	<0.0001	--	0.023	0.274	0.195	0.509
East Grand	<0.0001	<0.0001	<0.0001	--	0.298	0.216	0.524
Gaspereau	<0.0001	<0.0001	<0.0001	<0.0001	--	0.050	0.423
LaHave	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	--	0.338
blueback	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	--

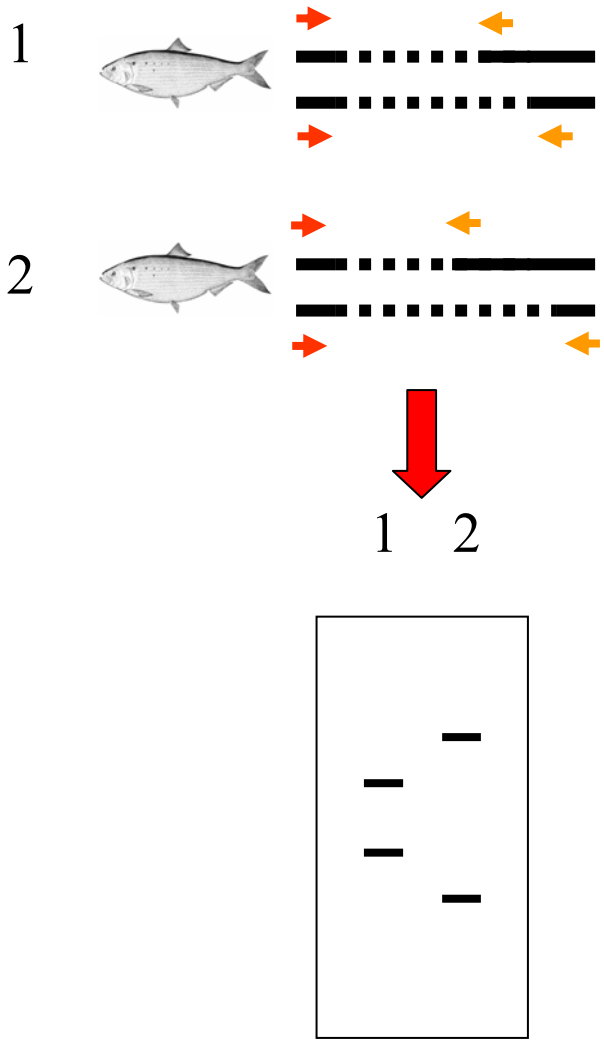

Figure 1. Schematic illustration of microsatellite genotyping. The dashed lines represent the repeat units of a microsatellite; the solid lines on either side correspond to the non-repetitive flanking DNA sequences. The arrows pointing right and left represent synthetic DNA primers that facilitate PCR amplification of the intervening sequences between the forward (left) and reverse (right) primers. In this example, each fish is heterozygous for this microsatellite; that is, the two allelic copies in each fish differ in the number of repeats. Following amplification, the amplified microsatellites are separated according to size by gel electrophoresis. Larger DNA fragments migrate more slowly on the gel, and appear higher on it. The size of the microsatellite alleles is determined by comparing their position on the gel to known DNA size standards.

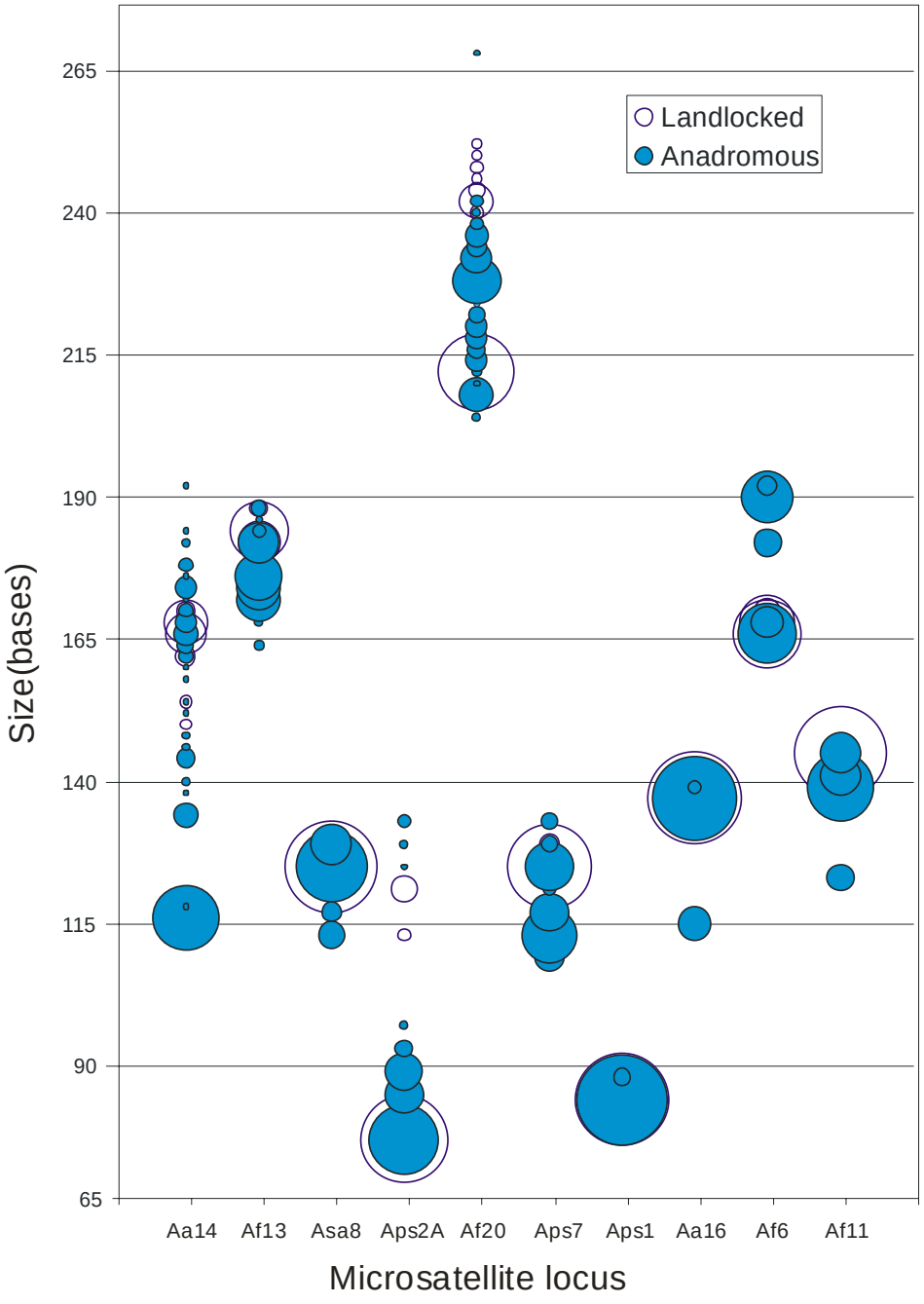

Figure 2. Frequency and size of microsatellite alleles in St. Croix alewives. Bubble sizes correspond to relative frequency of alleles. Values for landlocked and anadromous life history types are averages for the two population samples for each alewife form.

Figure 3. Estimated admixture proportions for individual alewives, assuming $k = 2$ clusters. Each fish is represented as a vertical bar; the colour(s) of the bar represent the different clusters (putative populations) that contribute to the genotype of each fish. Fish are grouped in the plot by population.

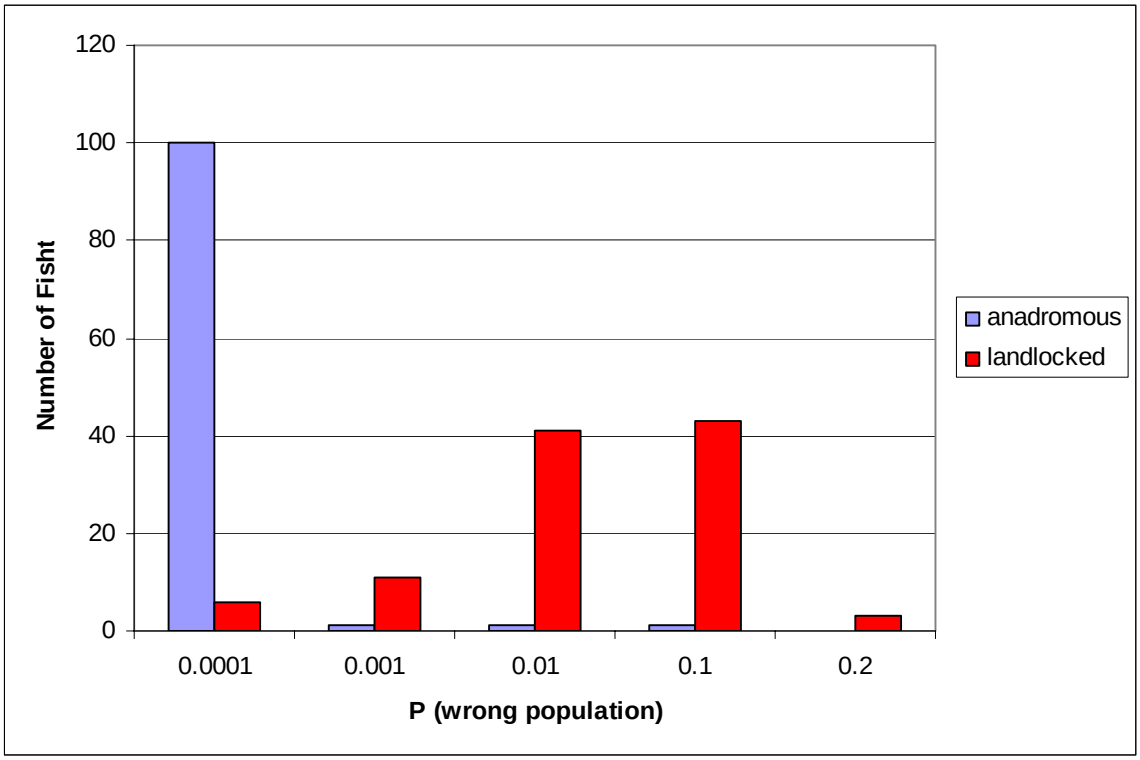

Figure 4. Frequency distributions for probabilities of St. Croix alewife genotypes in the 'wrong' population; that is, the probabilities that their genotypes could occur in the population in which they did *not* originate.

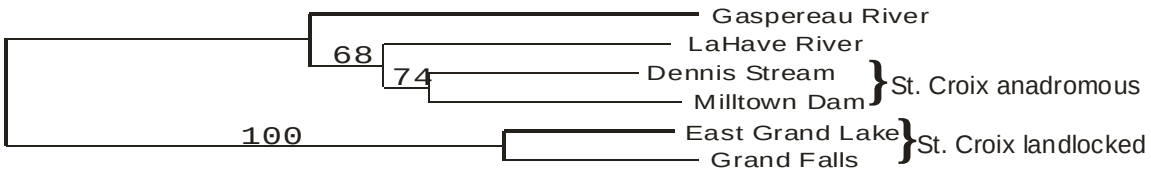

Figure 5. Dendrogram showing relationships of alewife samples. The tree was constructed using the neighbor-joining algorithm and Cavalli-Sforza Edwards genetic distances. The numbers on the branches indicate percent statistical support for particular clusters of populations.

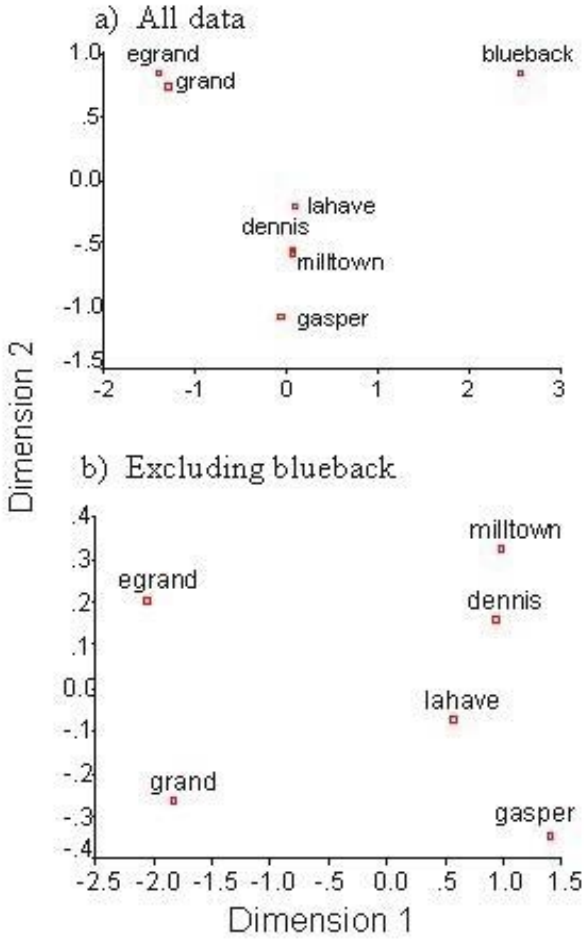

Figure 6. Multidimensional scaling plots showing the relationships of alewife populations based on pairwise estimates of F_{ST}, with and without blueback herring included.