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PHENOTYPIC DIVERGENCE OF INDIGENOUS AND TRANSLOCATED ARCTIC CHARR (*SALVELINUS ALPINUS*) POPULATIONS IN MAINE

By

Wendy K. Michaud

B.S. University of Maine at Farmington, 2002

A THESIS

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Master of Science

(in Zoology)

The Graduate School

The University of Maine

August, 2006

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By Wendy K. Michaud

Thesis Advisor: Dr. Michael T. Kinnison

An Abstract of the Thesis Presented
in Partial Fulfillment of the Requirements for the
Degree of Master of Science
(in Zoology)
August, 2006

Selection on traits related to trophic ecology is recognized as an important contributing factor in adaptive divergence and speciation. For several freshwater fish species, including Arctic charr (*Salvelinus alpinus*), such selection is commonly reflected in relationships between diet, habitat use and phenotypic divergence. Trophic specializations that emerge have been extensively studied among sympatric forms, but much less is known of the extent of this type of divergence in allopatry. Trait differences among these forms are also thought to reflect thousands of years of evolution, making it difficult to examine root causes of such divergence in natural populations. Here, I address the hypotheses that selection on trophic characters is important to incipient stages of divergence and the maintenance of specialized forms in allopatry, using indigenous and recently translocated populations of Arctic charr in Maine. To address this, I compared aspects of body shape, gill raker morphology, growth, and diet among six populations, including one transplant and its ancestral source. This examination revealed the presence of at least three trophic forms among Maine charr, including a benthic

specialist not previously identified in this region. Differences observed among these populations were analogous to those typical of trophic forms found elsewhere in sympatry, though perhaps less extensive in scale. Divergence between a translocated population and its source suggest some aspects of specialization are labile in contemporary time. In combination, these results indicate trophic ecology may play an important role in all stages of adaptive divergence, and niche stability may be important in maintaining trophic specializations over longer periods of time. In light of this new information, I also suggest that management plans for this species in Maine should seek to incorporate more information about such specialized forms, and should employ ecosystem based management to preserve forms within the unique contexts of their respective lake systems. Management approaches that fail to preserve lake community structure *in situ* are likely to result in either extinctions or revisions of specializations.

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CHAPTER 1 - GENERAL BACKGROUND

ADAPTIVE DIVERGENCE AND TROPHIC POLYMORPHISM

The process by which speciation occurs is perhaps one of the most debated topics in evolutionary biology, and the theory of "ecological speciation" is currently one of the most frequently investigated concepts in this field (see *TREE* special issue: Speciation, 2001). This theory suggests reproductive isolation could arise as a consequence of divergent natural selection on resource use (Dobzhansky 1946; Schluter 1996). According to this concept, divergent selection acts primarily on ecologically important traits as individuals exploit different niches within contiguous or separate ecosystems. Over time, populations adapt to the unique aspects of their environment and reproductive isolation builds as phenotypic and genotypic variation between groups increases (Schluter 2001). When reproductive isolation is complete, these ecologically distinct forms would fit the description of separate species as defined by the biological species concept (Mayr, 1942).

The study of ecological speciation has ultimately come to be dominated by studies of populations diverging in sympatry over postglacial time scales (reviewed by Schluter 2001). These cases do draw attention to the roles resource use and incipient reproductive isolation play in such divergence; however, there is little reason to suspect adaptive divergence is predominantly a sympatric process. Allopatric populations should theoretically also specialize on different resources because they are isolated in systems likely differing in many ecological factors. In addition, it is difficult to infer rates and mechanisms of incipient speciation in populations that have diverged over

postglacial time scales. In this thesis I suggest much can be learned from revisiting adaptive divergence in allopatric populations, and through examining this process over contemporary time scales.

Among the most widely recognized examples of adaptive divergence related to resource use are those of trophic polymorphisms associated with finding, capturing, and consuming specific prey items (reviewed in Skulason & Smith 1995). Again, most documented examples of trophic polymorphism are found among sympatric ecologically distinct "morphs" or "forms," presumed to represent diverging populations. For example, variation in beak size and shape found among Darwin's finches (*Geospiza* spp.) has been shown to be related to the size and hardness of seeds they consume (Grant 1986), and the amazing diversity seen among cichlids found in the African rift lakes can be correlated with their equally diverse diets (Greenwood 1984; Sage & Selander 1975). Trophic polymorphisms have also been noted in a wide variety of fishes found in recently glaciated northern lakes, including threespine stickleback (*Gasterosteus aculeatus*) (Schluter & McPhail 1992, Lavin & McPhail 1986), pumpkinseed sunfish (*Lepomis gibbosus*) (Robinson et al. 1993), lake whitefish (*Coregonus clupeaformis*) (Bodaly 1979; Bernatchez & Dodson 1990), rainbow smelt (*Osmerus mordax*) (Taylor & Bentzen 1993) and Arctic charr (*Salvelinus alpinus*) (reviewed in Jonsson & Jonsson 2001).

The results of trophically related selection are often manifest in morphological, life history, and behavioral differences among forms (Skulason & Smith 1995; Schluter 1996; Webb 1984). For freshwater fish, as in many other species, these patterns are strongly related to both the type of food in their diet as well as the area in which prey

are found. Fish feeding on larger or harder prey items tend to have larger, more robust jaws, and fewer, stubbier gill rakers. Other features are associated with the habitat and method by which a particular form forages. For example, fish seeking prey in open water tend to have more streamlined bodies, pointed snouts with terminally oriented mouths, and shorter fins. Finally, life history characters also tend to diverge in association with availability, energy content, and size of a particular prey type. For instance, piscivores often exhibit a faster growth rate and are older and larger at maturity than planktivores. Such trophic-related differences in morphology, ecology, and life history are well characterized in the Arctic charr (reviewed in Jonsson & Jonsson 2001).

Trophic polymorphism in charr is typified by the coexistence of a "normal" benthivorous or piscivorous form and a "dwarf" planktivorous form residing in the same lake (Jonsson and Jonsson 2001, Schluter, 1996, Skulason & Smith 1995). Researchers have repeatedly demonstrated a strong link between phenotypic differences found among these forms and their trophic specializations (reviewed in Jonsson & Jonsson 2001). Benthic forms typically feed on macroinvertebrates (usually mollusks), have a subterminal mouth, and larger fins. Pelagic forms tend to have a terminal mouth, smaller paired fins and a more streamlined body. Life history traits also appear to reflect these specializations, with piscivorous forms typically exhibiting larger size at a given age as well as higher reproductive investment than their benthic or planktivorous counterparts, (a presumed result of the higher energy content of their prey).

The prevalence of polymorphism in Arctic charr (and other freshwater fishes) is often attributed to the availability of open niches and lack of interspecific competition

in recently deglaciated lakes (Robinson & Wilson 1994, Skulason & Smith 1995, Jonsson & Jonsson 2001). By exploiting these open niches, specialists are able to escape intraspecific competition for limited food resources. Lake Thingvallavatn, Iceland, provides an extreme example of potential sympatric divergence attributable to just such processes (Sandlund et al. 1992). Here piscivorous, planktivorous, and two benthivorous, forms coexist within Iceland's largest lake, (figure 1.1), where the availability of alternative habitats in such a large ecosystem could have favored the coexistence of so many forms. Is intraspecific competition necessary to promoting and maintaining these specialist forms? A study of adaptive divergence among allopatric populations may partly address that question, if these specialized forms develop in the absence of competition from one another.

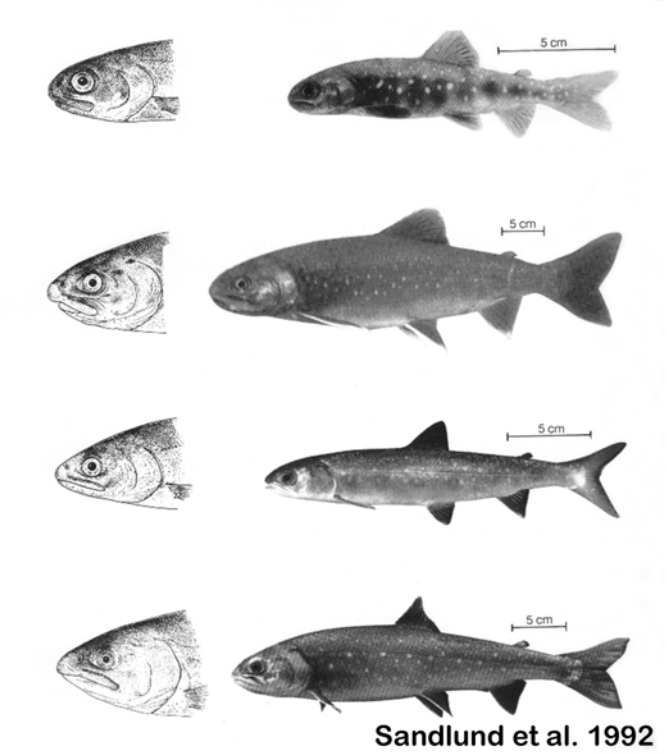

Figure 1.1. Four trophic forms of Arctic charr in Thingvallavatn, Iceland, (from Sandlund et al. 1992). From top: small benthivore, large benthivore, small planktivore, large piscivore.

EVOLUTION IN CONTEMPORARY TIME

Addressing the root causes of evolution in wild populations is often an indirect exercise, because data are usually available only from populations thought to have diverged over many thousands of years. As a result, it is frequently presumed factors that drove divergence in the past are comparable to those reinforcing divergence in the present. Likewise, a resolution of thousands of years may be too coarse to appreciate the rates at which such diversity arises. Yet situations do exist for more directly studying the initial mechanisms behind adaptive divergence. One such opportunity is afforded as an interesting byproduct of the centuries-old legacy of intentional and accidental translocation of organisms by humans.

When records of sources and times of introductions are available, one has the ability to examine both the tempo and mode of evolution over very short time scales (e.g. Reznick et al. 1997; Hendry et al. 2000; Kinnison et al. 2001; Stockwell & Weeks 1999). Since translocation records are usually only available from the recent past, these populations are likely to be in the beginning stages of divergence. Changes observed between a transplant population and its source, or among transplant populations, can be documented and studied nearly as they occur, and need not be inferred from later morphological or genetic reconstruction. In addition, because time of separation is known, rates of divergence can be estimated directly (Hendry and Kinnison 1999). This information may provide some insight into the processes involved in adaptive divergence that cannot otherwise be observed in many wild populations. Although there have been many studies of the evolution of exotic fish populations, (Kinnison and

Hendry 2001), I am not aware of such a study involving the rapid evolution of trophic specializations in wild fish populations.

ARCTIC CHARR IN MAINE

Landlocked Arctic charr populations can be found throughout northern Europe, Asia and North America. Mitochondrial DNA work conducted by Brunner et al. (2001) identified 5 genetic lineages within the species, the geographically most restricted of which is the Acadian. This lineage is comprised of populations from southern Quebec, New Brunswick, and Maine. Currently, only 12 indigenous populations of this species can be found in Maine (figure 1.2), and they represent both the southern-most populations of the species in North America, and the only indigenous Arctic charr found in the United States outside of Alaska (Kircheis 1989; Frost 2001). This species was formerly found in Vermont and New Hampshire, but disappeared in the late nineteenth century along with at least one Maine population (Kendall 1914). This extirpation was most likely due to the introduction of other predatory and competitor species in these systems.

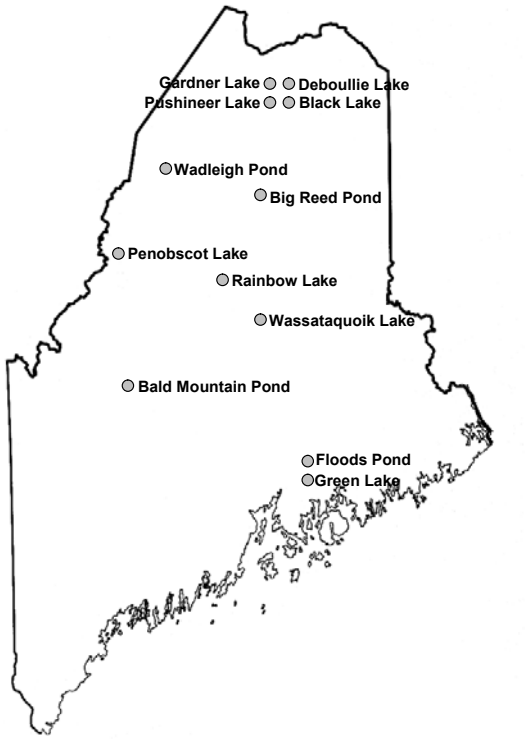

Figure 1.2. Maine lakes containing indigenous Arctic charr populations.

Little has been published on the evolution and ecology of Maine's Arctic charr (Everhart 1950; Waters 1952; Kircheis 1976; Kircheis 1980). Nonetheless, anecdotal evidence suggests trophic divergence may exist among these populations. Anglers and biologists historically distinguished between local charr populations, and recognized two subspecies of Arctic charr in Maine (Kendall 1914): the "blueback trout," *Salvelinus oquassa*, (Bean 1887) and the "Sunapee" or "Silver trout," *Salvelinus aureolus* (Girard 1854). Traditionally, bluebacks were typified by populations in northern and western Maine, and named for the dark-bluish color of their backs during spawning. They were regarded as relatively small, planktivorous forage fish for the much larger eastern brook trout (*Salvelinus fontinalis*) with which they commonly coexist. The Sunapee form was characterized as a larger, piscivorous fish sought in its own right as a game species, especially in Sunapee Lake, New Hampshire, for which the form is named. Unfortunately, such a form is now thought to remain only in Floods Pond, Maine (Kircheis 1976).

Interest in protecting the rare Sunapee form led to several genetic studies of divergence among Arctic charr in Maine. These studies were conducted primarily to address the question of whether the blueback and Sunapee forms represented unique genetic lineages that perhaps invaded lakes in Maine from different glacial refugia. A study examining variation in mitochondrial DNA by Kornfield and Kircheis (1994) included samples from the Sunapee population in Floods Pond as well as two blueback populations from northern Maine. Each population was found to have a different restriction fragment phenotype, which did not support the hypothesis of monophyletic Sunapee and blueback lineages. Following this, Bernatchez et al. (2002) published the

results of a study comparing six microsatellite loci among samples from all twelve indigenous populations of Arctic charr in Maine, including Floods Pond. Highly significant differences in allelic frequencies were found among most samples (mean pairwise Fst = 0.092, range 0.032-0.171), but again, no evidence was found to suggest bluebacks were monophyletic relative to the charr in Floods Pond. In fact, no clear patterns of genetic divergence related to drainage or overall geography (with the exception of populations in two physically connected lakes) were uncovered, suggesting most populations have been isolated from each other for thousands of years.

Prior to these genetic studies, Arctic charr from Floods Pond were translocated into several other lakes as part of the Sunapee trout conservation effort (Kircheis 1989). Floods Pond, which is the water supply for the greater Bangor area, experienced severe water draw-downs in the 1970's. During these periods, water levels dropped below the primary spawning area used by the Arctic charr. As the threat to this rare fish became apparent, the Maine Department of Inland Fisheries and Wildlife responded by transplanting these charr into 7 other systems throughout Maine. Of these, naturally reproducing populations persist in only two lakes; Long Pond and Enchanted Pond (Frost 2001). Although these fish have not been examined in great detail since their introduction, other studies of translocated organisms suggest these fish have the potential to diverge from the ancestral form they were meant to conserve (Sockwell & Leberg 2002).

Arctic Charr populations in Maine thus present the rare opportunity to study adaptive divergence in allopatric populations over both glacial and contemporary periods. Anecdotal information on differences in body shape, color, and diet from

biologists and anglers indicate Maine populations may indeed exhibit a variety of trophic specializations like their sympatric counterparts. If so, examining such divergence may provide insight into the relative importance of sympatry as a driving factor in trophic polymorphisms and speciation. Moreover, well-documented translocations of the species may help address the lability of such specializations, as well as the incipient tempo and mode of trophic polymorphisms and ecological divergence.

OBJECTIVES

The main goals of this thesis are: 1) to determine whether indigenous Arctic charr populations in Maine differ in morphology and life history phenotypes; 2) to assess whether such patterns of divergence are correlated with trophic specializations; 3) to determine if translocated Arctic charr exhibit phenotypic divergence over contemporary time periods; and 4) to determine whether contemporary divergence follows patterns of trophic specialization consistent with those described for longer, postglacial, time scales.

The second chapter of this thesis focuses on the first and second objectives, which address the general question of whether postglacial divergence in allopatry follows patterns previously described for sympatric forms of this species. This includes an evaluation of the aforementioned anecdotal evidence for trophic related trait variation among Arctic charr in Maine, which will allow us to determine if two forms do exist and to better quantify differences between them. Chapter three addresses the third and fourth objectives, which are aimed at further refining the question of

ecological divergence to consider the rate at which trophic specializations might arise in the wild. Combined, these chapters are largely targeted at the academic goal of understanding the role of trophic specialization in divergence and speciation. However, many of the results of this work are also important for refining management plans of this rare species in Maine. Therefore, the final chapter of my thesis addresses the management and conservation implications of this research.

CHAPTER 2 - ECOLOGICALLY DRIVEN DIVERGENCE AMONG INDIGENOUS ARCTIC CHARR POPULATIONS IN MAINE

ABSTRACT

Selection on traits related to trophic ecology is recognized as an important factor in adaptive divergence and speciation. For freshwater fish species, trophic specializations have been extensively studied among sympatric forms, but much less is known of the extent of this divergence in allopatry. Anecdotal descriptions of two Arctic charr forms in the state of Maine, USA, suggested divergence among these allopatric populations may parallel trophic specializations observed for sympatric forms. I addressed this hypothesis by examining aspects of body shape, gill raker morphology, growth, and diet for five of these populations. Comparisons indicate significant diet differences and trait divergence among populations, and revealed the presence of at least 3 trophic forms analogous to those typical of sympatric systems. The scale of divergence appears to be less extreme among these populations than previously documented for other systems, perhaps due to reinforcing processes found only in sympatry.

INTRODUCTION

Trophic ecology is thought to be an important factor in the generation of intraspecific and interspecific diversity (Skulason & Smith 1995; Schluter 2000). This form of adaptive divergence is driven by selection on characteristics related to finding, capturing, and consuming specific prey items, and is commonly cited as a factor in models of ecological speciation (Schluter 1996, 2000). Perhaps some of the best known

examples of this type of divergence are represented by trophic polymorphisms, in which ecologically distinct "morphs" or "forms" coexist in the same lake (Skulason & Smith 1995). Such polymorphisms have been widely noted among fishes found in recently deglaciated northern lakes, including threespine stickleback (Schluter & McPhail 1992, Lavin & McPhail 1986), pumpkinseed sunfish (Robinson et al. 1993), lake whitefish (Lindsey 1981; Bernatchez & Dodson 1990), rainbow smelt (Taylor & Bentzen 1993) and Arctic charr (reviewed in Jonsson & Jonsson 2001). Models for the origins of such specialists often emphasize the role of intraspecific competition in favoring the evolution of these polymorphic populations from generalist ancestors (Robinson & Wilson 1994; Skulason & Smith 1995; Schluter 2000). In this study I consider trophic specialization in allopatric populations of Arctic charr and the broader role of trophic ecology in the evolution of diversity outside of sympatry.

Comparisons of trophic specialization in allopatry and sympatry may help address the root causes and maintenance of such adaptations. For example, release from intraspecific competition is often cited as a major factor favoring sympatric divergence (Robinson & Wilson 1994; Skulason & Smith 1995; Schluter 2000), however, this type of competition would not play a role in divergence among allopatric populations. If trophic specializations are very similar in pattern and scale in allopatry and sympatry, then one might look toward other factors favoring specialization, such as patterns of resource availability or the effects of interspecific competitors. Likewise, studies of allopatric divergence could provide insight into the scope for alternative models of coexistence involving elements of ancestral allopatry and secondary contact.

Typical trophic specializations extend well past differences in foraging behavior. For freshwater fishes, the morphology of different trophic specialists is strongly correlated with the food items they consume as well as the habitat in which their prey is found (Skulason & Smith 1995; Schluter 1996; Webb 1984). In direct relation to prey size and hardness, divergent forms commonly differ in jaw shape and size, as well as the shape and number of their gill rakers. Other morphological features, such as overall body, head, and fin proportions, are commonly associated with the habitat and style in which a particular form forages for food (Webb 1984). Finally, life history characters such as growth, age, and size at maturity are also commonly associated with the availability, energy content, and relative size of dominant prey items. Such differences are very well documented among sympatric forms of Arctic charr (reviewed in Jonsson & Jonsson 2001), yet very little is known of the scope of allopatric specialization in this species.

Anecdotal evidence suggests trophic specializations may have evolved in allopatry among the 12 remaining indigenous populations of Arctic charr found in Maine, USA (Kircheis 1989; Frost 2001). Historically, biologists and anglers recognized two distinct forms in the state; the "blueback" and "Sunapee" or "silver trout" (Kendall 1914), even attributing them species status (Bean 1887; Girard 1854). Charr in northern and western Maine waters are commonly referred to as blueback trout because of the dark-bluish color of their backs during spawning (Kircheis 1980). These fish were generally regarded as relatively small, planktivorous, forage fish for the piscivorous brook charr with which they commonly coexist. The Sunapee trout is characterized as a larger, piscivorous fish, once sought after as a game species in its

own right. Currently, the only remaining indigenous population of this form is thought to occur in Floods Pond, located in central Maine (Kircheis 1989). However, mitochondrial DNA (Kornfield & Kircheis 1994) and microsatellite (Bernatchez et al. 2001) evidence suggest the blueback form is not monophyletic with respect to the Sunapee form in Maine. This suggests the forms are conspecific, though nearly all charr populations in Maine are genetically distinct (mean pairwise Fst = 0.092, range 0.032-0.171) (Bernatchez et al. 2001).

I propose, based on this evidence, that the blueback and Sunapee forms represent trophic specialists that have evolved postglacially in allopatry. The specific objectives of this study are: 1) to formally determine whether variation in morphology and life history exists among indigenous Arctic charr populations in Maine; 2) to assess whether such patterns of divergence are correlated with trophic ecology; and 3) to qualitatively compare the divergence among these allopatric populations with patterns of divergence previously described for sympatric populations of this species.

METHODS

Sample Collection

Arctic charr were sampled from Floods Pond, Gardner Lake, Penobscot Lake, and Wadleigh Pond during July of 2003 and 2004 (table 2.1, figure 2.1). Fish were captured using monofilament gillnets (mesh size ½"-1" stretch) set at depths between 14 and 30m for 12-24 hours. Also included in this study is a sample of charr collected from Rainbow Lake, captured by hook and line during March of 2004 and 2005. These samples include four nominative blueback populations as well as the Sunapee

population from Floods Pond. In each case, fish were placed on ice immediately after capture and photographed within 12 hours. The left side of each fish was digitally photographed at a fixed focal length, with a ruler included for size reference. Caliper measurements, including head width (measured as interorbital distance) and head depth (measured at the posterior edge of the skull), as well as weight, were also recorded at this time. After initial processing, individuals were frozen until they could be dissected. After thawing, gill arches, sagittal otoliths, and stomach contents were removed from each fish. Sex and maturity status were also assessed by gonadal examination. Morphological analyses used only fish identified as females, in order to avoid confounding trophic divergence with differences in sex ratios and the pronounced secondary sexual trait development observed in mature males.

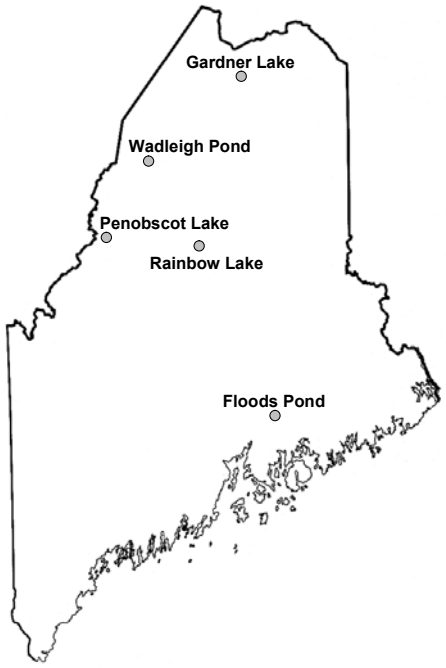

Figure 2.1. Map showing locations of Arctic charr populations included in this study

Table 2.1. Sample locations of Sunapee and blueback forms, with abbreviations, drainages of origin, lake area and depth, and sample sizes of both females and males obtained.

Lake	Abb.	Drainage	Area (hectares)	Mean (max) Depth (m)	Sample Size
Sunapee					
Floods Pond	**FLP**	**Union**	**257**	**12 (45)**	**28♀ 15♂**
Blueback					
Gardner Lake	**GDL**	**St. John**	**115**	**12 (37)**	**18♀ 18♂**
Penobscot Lake	**PNL**	**Penobscot**	**470**	**10 (32)**	**46♀ 39♂**
Rainbow Lake	**RBL**	**Penobscot**	**658**	**12 (40)**	**19♀ 22♂**
Wadleigh Pond	**WDP**	**Penobscot**	**65**	**6 (14)**	**25♀ 24♂**

Trophic Ecology

Stomach contents were removed from all sampled individuals and examined under a dissecting scope to determine the presence of zooplankton, insect larvae or pupa, benthic invertebrates (including gastropods, bivalves, and amphipods), and fish. Differences in the frequency with which prey items were found in stomach contents among populations were assessed using a Pearson chi-squared test for each prey category.

Gill Raker Morphology

The most anterior, left gill arch was extracted from each fish sampled (both males and females), rinsed, and placed in ethanol to prevent decay. Individual arches were pressed flat between sheets of plexiglass, and photographed at a fixed focal length along with a ruler for size reference. From these images, measurements of mean gill raker length, width at the base, and spacing (measured from the edge of one raker to another at the base), were obtained for the first three rakers below the apex on the ventral side of the arch, using the program ImageJ version 1.32 (Rasband 2004). The

number of gill rakers per arch was also counted by examining these images. The mean number of gill rakers per arch, gill raker length, width, and spacing were compared among populations using analysis of variance.

Growth

Sagittal otoliths were removed from each fish for age determination. To facilitate reading of annuli under a dissecting scope, each otolith was placed whole in a small glass dish containing 50% glycerin solution and illuminated from the side using a fiber optic light source. Annuli were counted on both left and right otoliths (when available), and age was determined based on agreement between these counts.

Population growth curves were estimated using the Von Bertalanffy growth function (VBGF): $L_t = L_\infty(1-e^{-k(t-t0)})$ where L_t is the length at age t, L_∞ is the asymptotic length for the population, k is the Brody growth coefficient, and t0 is the age at which length = 0 (Ricker 1975). This model was fitted to length at age data for each population using a combination of the best fit model approach and biological criteria, because there were so few data points for very young fish. These growth curves were compared among all populations using the analysis of residual sums of squares method (Chen et al. 1992).

Morphological Analysis

Standardized digital images of each fish were used to examine variation in body shape. Eighteen homologous landmarks were placed on each image (figure 2.2) using the program tpsDig, version 2.0 (Rohlf 2004). Individuals were analyzed in random

order within site by the same person. The coordinates of these landmarks were then uploaded into tpsRelw, version 1.42 (Rolf 2005) to calculate relative warp scores for each fish. This program first aligns each specimen to a generalized orthogonal least-squares Procrustes consensus configuration to remove isometric effects of body size from the analysis. Next, orthogonal partial warps are computed using a thin-plate spline technique to explain shape deformations in the x and y planes. The variation in shape is then summarized in relative warps, which are essentially calculated as principal components based on the partial warp scores. Relative warp scores were obtained using warp functions derived from a larger database of Arctic charr collected in Maine (n=335), including both males and females, to provide a maximally robust assessment of shape variation in Maine charr. An additional population (Long Pond) was also included in this database to ensure comparability with a related study on trophic specializations in translocated populations (see chapter 3). Relative warp scores for females from the five populations considered here were then included in a discriminant functions analysis to assess the full multivariate divergence in shape among populations. The resulting discriminant functions were regressed back onto the principal warps, using tpsRegr (version 1.31 - Rohlf 2005), to aid interpretation.

In addition to relative warp scores, our analysis of morphology also included a series of linear measurements, including fin lengths (with the exception of the adipose fin), body length (measured from the anterior of the eye socket to the posterior most point of the caudal peduncle), eye width, maxilla length, and interorbital distance. Interorbital distance was measured using calipers and will also be referred to as head width. All other measures were derived from digital photos and adjusted to a common

body size of 188.96 mm across all populations using ANCOVA. Size corrected measures were then subjected to a principal components analysis to reduce the data to a set of factors that best describe variation relative to body shape.

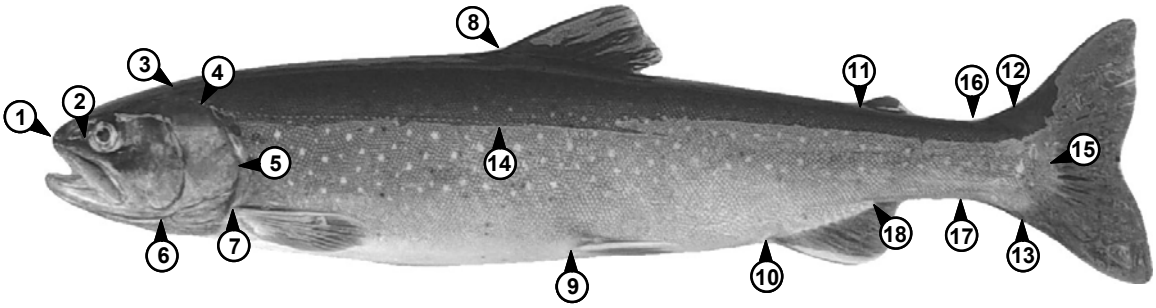

Figure 2.2. Anatomical landmarks used for morphological analyses. These include: 1) tip of snout; 2) left of eye socket; 3) posterior edge of skull; 4) top of operculum; 5) most posterior point of operculum; 6) bottom of operculum directly below lower most point of preoperculum; 7-13) fin insertions; 14) point on lateral line directly below insertion of dorsal fin; 15) posterior center point of caudal peduncle; 16&17) narrowest part of caudal peduncle; and 18) insertion of last anal fin ray.

All statistical analyses presented here were run using SYSTAT, version 11 (2004) unless otherwise noted.

RESULTS

Trophic Ecology

The majority of fish from each lake consumed insect larvae, but each population differed significantly in the inclusion of other prey items in their diet (table 2.2). Organisms identified as zooplankton included mainly cladocerans and copepods; the insect category was comprised almost entirely of diptera larvae; benthic prey items included amphipods, gastropods and bivalves; and fish that could be identified included rainbow smelt, threespine stickleback, pumpkinseed sunfish, and small charr.

Generally, items from all four prey categories were found among the stomach contents of fish from each lake, with the exception of fish in Wadleigh Pond, benthic items in Floods Pond, and zooplankton in the Rainbow Lake sample. It should be noted this lack of zooplankton in the diet of Rainbow Lake charr may be due to the decreased abundance of this prey item during the winter months, when these fish were captured. Nevertheless, the frequency with which individuals included prey items other than insect larvae in their diet still varied significantly among populations (table 2.2).

Table 2.2. Percent individuals from each population with a given prey category present in its stomach contents. Numbers in parentheses represent the number of individuals from which stomach contents was obtained for each population. P-values were obtained from Pearson's chi-squared test.

	plankton	Insect	benthic	fish
FLP (38)	3%	76%	0%	50%
GDL (40)	3%	75%	95%	5%
PNL (82)	2%	98%	6%	23%
RBL (31)	0%	81%	42%	10%
WDP (45)	13%	98%	7%	0%
P-value	**	< 0.001	< 0.001	< 0.001

** insufficient data for significance test

Gill Raker Morphology

All measured aspects of gill raker morphology differed among populations. The number of gill rakers differed significantly among populations ($p = 0.011$), with Penobscot lake averaging the fewest (15.23 ± 0.45) and Wadleigh Pond the most (16.39 ± 0.5). Floods and Penobscot fish had the longest mean gill raker length, while those in Gardner had the smallest ($p < 0.001$). The average width of gill rakers also differed among populations ($p < 0.001$), with Floods, Penobscot, and Rainbow Lake charr having the widest gill rakers and Gardner the thinnest. Gill rakers of charr from

Penobscot Lake exhibited the largest mean spacing, while those from Gardner Lake averaged the most densely packed rakers ($p < 0.001$) (figure 2.3).

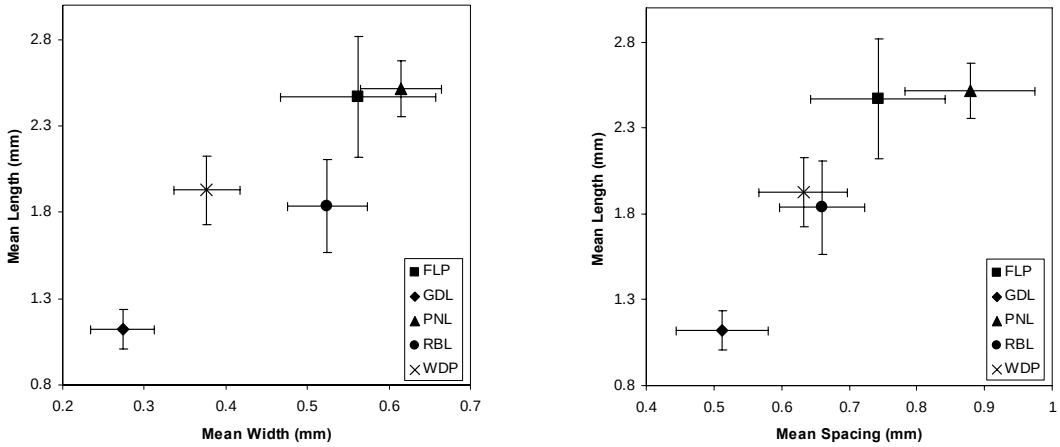

Figure 2.3. Population means for mean gill raker length, width, and spacing, with 95% confidence intervals.

Growth

Overall, samples from these populations overlapped in sizes and ages. Only Gardner Lake was smaller on average than Floods Pond ($p=0.001$), Penobscot Lake ($p=0.002$), and Rainbow Lake ($p=0.012$), while fish from Wadleigh Pond were younger than all other populations on average ($p<0.001$). Even so, the VBGF curves varied significantly among populations ($F_{14,120} = 102.533$; $p<0.001$) (figure 2.4). Charr from Floods Pond had the largest asymptotic length (L_∞) and the lowest k value, or the slowest growth towards L_∞. In contrast, the asymptotic length for Gardner Lake fish was the smallest,

Table 2.3. Von Bertalanffy Growth Function parameters for each population.

	$L\infty$	k	t_0
FLP	810.30	0.033	-1.869
GDL	182.23	0.880	2.483
PNL	776.23	0.036	-2.555
RBL	457.99	0.077	-1.137
WDP	353.96	0.153	-2.021

and this population had the largest growth coefficient value (table 2.3). It should be noted, however, that the values of L_∞ may be overestimated, and the values for k underestimated due to lack of very young and very old individuals in these samples.

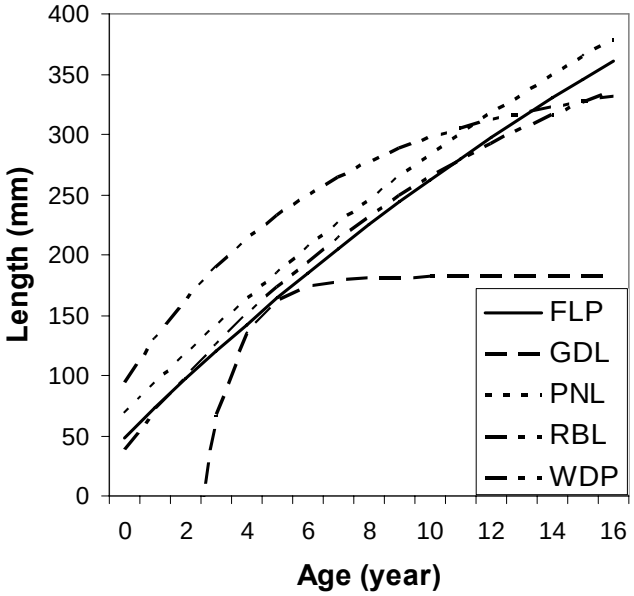

Figure 2.4. Growth curves for each population generated by the Von Bertalanffy model. (see Appendix for individual population size-at-age data)

Morphological Analysis

Linear measures included in this analysis were best summarized using three varimax rotated principal components. The variation explained by the first component was mostly accounted for by upper and lower caudal fin lengths (21% of total variation). The second component explained 26% of the total variation, which was mostly due to variation in anal, dorsal, pectoral, and pelvic fin lengths. Head width, eye width, and maxilla length all loaded highly on the third principal component, which

Table 2.4. Correlations between principal components and morphometric traits. Characters most highly correlated with each principal component are noted with an asterisk.

	PC1	PC2	PC3
Anal Length	0.046	0.847*	0.057
Dorsal Length	0.078	0.530*	0.471
Eye Width	0.285	0.331	0.588*
L. Caudal Length	0.869*	0.158	0.227
Maxilla Length	0.088	0.188	0.871*
Pectoral Length	0.430	0.696*	0.252
Pelvic Length	0.179	0.783*	0.156
U. Caudal Length	0.881*	0.170	0.177
Head Width	0.227	0.023	0.722*

explained 22% of the total variation (table 2.4). Combined, these three components explained 69% of the total variance in the data. Analysis of variance on these components revealed that only PC2 and PC3 significantly differentiated between populations (PC1: $p = 0.1065$, PC2: $p < 0.001$, PC3: $p < 0.001$). Charr from Gardner Lake had the longest fins and those from Wadleigh had the shortest, while Floods and Gardner charr had the largest head features and those from Rainbow and Wadleigh had the smallest (figure 2.5).

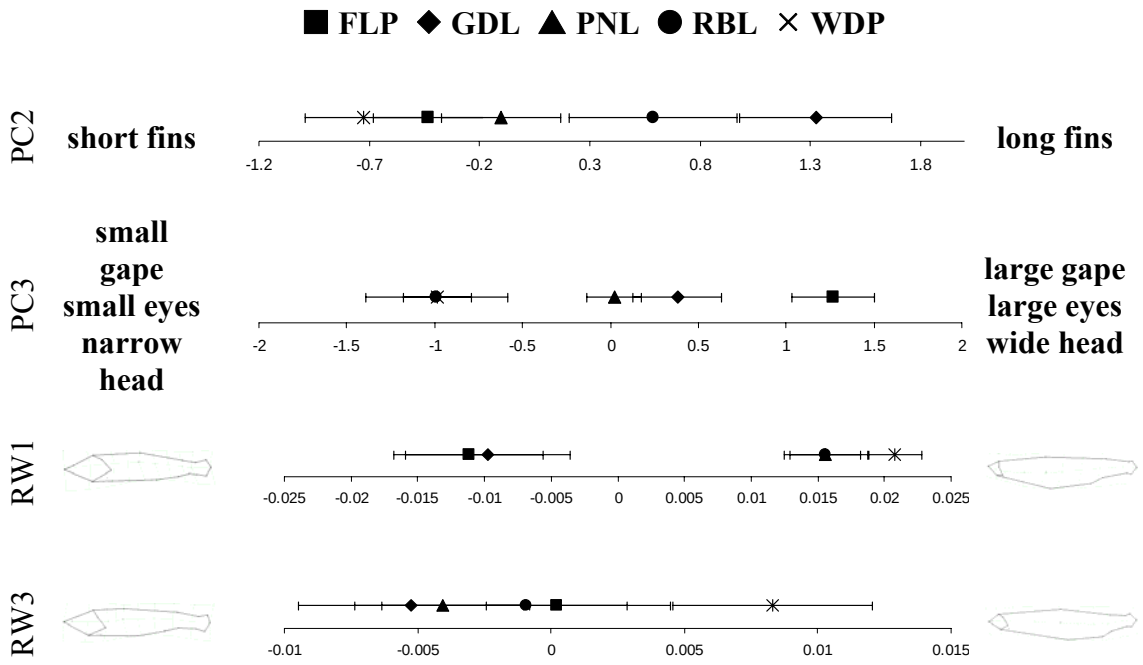

Figure 2.5. Mean principal component (PC) scores and relative warp (RW) scores for each population (± 95% CI). Interpretations of high negative and positive principal component scores are indicated at each end of the axis. Transformation grids show extremes of the shape variation associated with each relative warp function (relative warp two is not shown as it is associated with variation due to positioning of fish in photos). These visualizations have been exaggerated three-fold to aid interpretation.

Discriminant analysis on all relative warps produced four functions that significantly differentiated among the populations (Wilks $\Lambda = 0.008$, Approximate $F_{(128, 400)} = 7.467$, $p < 0.001$). The reclassification rate also indicates strong differences in body morphology exist among groups (overall jackknifed classification rate = 82%), with charr from Wadleigh having the highest reclassification rate (96%), and Rainbow the lowest (58%). Variation along discriminant function one appears to be related to variation in overall head size, length and width of the caudal peduncle, body depth, and position of fin insertions, as visualized by the thin-plate spline depictions of the discriminant functions (figure 2.6). This function is highly correlated with relative warp one (r=0.763) which describes variation in head size and body depth, as well as distance between the anal and adipose fin insertions and the caudal fin insertions (peduncle length) (figure 2.5) Discriminant function two revealed differences in overall body depth and location of fin insertions. This was correlated with relative warp three (r=0.309), which explains variation in head size, body depth, as well as the relative length of the body anterior of the dorsal fin to length posterior to the dorsal fin. Combined, the first two discriminant functions explain 72% of the total dispersion of the populations.

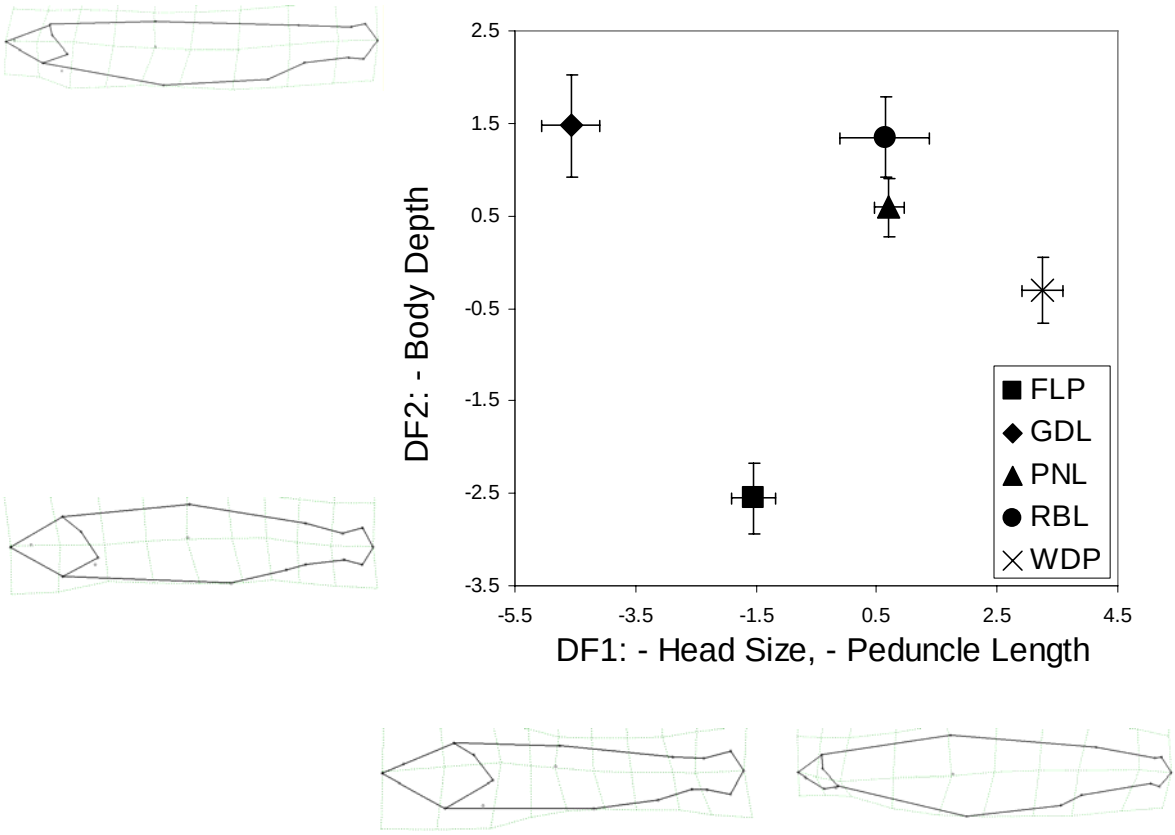

Figure 2.6. Shape variation among populations of arctic charr. Plot shows mean discriminant function scores for each population with 95% confidence intervals. Axis labels indicate features strongly associated with each discriminant function, and the direction of this relationship is indicated by a "+" or "-" sign. Transformation grids show extremes of the shape variation associated with each function, created by regressing each discriminant function back on the partial warp scores. These visualizations have been exaggerated three-fold to aid interpretation.

DISCUSSION

The results of this study indicate substantial phenotypic divergence exists among the five allopatric Arctic charr populations considered here. Significant differences in diet, body shape, gill raker design, and growth were detected. In combination, these phenotypic differences appear to be comparable to the trophic specializations of sympatric forms noted elsewhere (Jonsson & Jonsson 2001). Surprisingly, our data

suggest the existence of at least three specialized forms of Arctic charr in the state of Maine: a large pelagic piscivore, small benthivore, and small pelagic insectivore/planktivore.

There is clearly substantial evidence to corroborate the hypothesis that the "Sunapee" population in Floods Pond represents a piscivorous specialist. Consistent with their diet, these charr have relatively large, widely spaced gill rakers, a design noted elsewhere for piscivores (Snorrason et al. 1994; Alekseyev et al. 2002). Morphologically, these fish exhibit the large head and wide gape commonly associated with piscivore, as well as the shorter fins and large eyes of a pelagic predator that cruises the water column in search of quick moving prey (Webb1975; Walker 1997). These charr also grow to a relatively large asymptotic size, perhaps as both a consequence and adaptation for feeding on larger prey.

Anecdotal descriptions of the blueback form suggested it would be a small, pelagic, planktivore. However, not one of the purported blueback populations sampled here utilized zooplankton as a primary adult food source during the time they were captured. Furthermore, significant differences in diet and several trophic related characters were noted among these populations, with the greatest axis of divergence found between apparent pelagic insectivores and benthivores.

The discovery of a blueback population of benthic specialists in Gardner Lake was particularly unexpected, because this form was not previously reported among charr in Maine. Diets of Arctic charr from this population were clearly dominated by benthic food sources, including small bivalves, gastropods, and amphipods, which was apparent not only from stomach contents but morphological differences as well. These

charr had much smaller gill rakers, and a relatively large mouth gape compared with other populations. Such characteristics are consistent with the observation that mollusks were apparently dislodged from the substrate and swallowed whole by these fish. This strategy would be different than that employed by other molluskivores, such as sunfish (*Lepomis* spp.) or certain cichlids (e.g. *Cichlasoma* spp.) that crush their prey and sort edible parts, shell fragments and substrate through better developed and spaced rakers (Lauder 1983; Meyer 1989). Gardner Lake charr also had large eyes, which could be useful in spotting cryptic invertebrates against substrates in the littoral zone. These fish exhibited the relatively large fin size and thick caudal peduncles typical of benthic or littoral forms, which may improve maneuverability in a structurally complex environment (Webb 1984; Snorrason et al. 1994; Walker 1997). Finally, this population of Arctic charr has an initially faster growth rate, and reaches a smaller asymptotic body size than observed in the other lakes. This may be attributed to the relative abundance of this prey source in a very unproductive lake with such a narrow littoral zone.

Though all populations consume aquatic insect larvae and pupae, Wadleigh Pond charr do not appear to include fish or benthic items as frequently as other populations do, indicating they may specialize on this resource. Their phenotypic characters are typical of those observed for small pelagic forms in other populations of Arctic charr (Fraser et al. 1998; Snorrason et al. 1994; Alekseyev et al. 2002). These fish have long, closely spaced gill rakers typically associated with a higher degree of planktivory and reliance on smaller food particles (Magnuson & Heitz 1971; Sanderson et al. 1991). Charr from Wadleigh Pond also have the typical pelagic body form, with

smaller fins, a slender body, and thinner caudal peduncle thought to increase efficiency during sustained cruising through open water in search of patchily distributed prey (Webb 1975; Walker 1997). Finally, they also reach a smaller asymptotic length and have a faster initial growth rate than populations that include fish in their diet (eg. charr from Floods Pond and Penobscot Lake).

The remaining two populations, Penobscot and Rainbow, appear to have more mixed diets; neither showing evidence of a clear ontogenic niche shift. Both included insect larvae, fish, and benthic macroinvertebrates in their diet, with Rainbow Lake charr feeding more heavily on mollusks and Penobscot Lake charr feeding more often on fish. Consistent with this, these populations showed intermediate traits with respect to the more specialized forms. In general, their body morphology was somewhat analogous to fish from Wadleigh Pond, with relatively small heads, streamlined bodies, and thinner caudal peduncles. However, Rainbow Lake fish had relatively longer fins and a body shape approaching the design of benthivores from Gardner (figures 2.5 & 2.6.). This is consistent with the observation that these fish consumed more benthic invertebrates than any other population studied besides Gardner. Likewise, Penobscot charr consumed more fish than any population other than Floods, with which they overlapped in gill raker size and spacing.

The specific traits that differ among trophic specialists appear to be very similar in both allopatry and sympatry. This supports the hypothesis that aspects of trophic ecology play a primary role in driving adaptive divergence among populations in this species, regardless of other ecological factors involved. However, the scale of divergence between specialized forms appears to be greater in a number of sympatric

systems than what I observed for Maine charr (e.g. no divergence approached the scale found in Thingvallavatn – figure 1.1). This would be consistent with models emphasizing a role for intraspecific competition in favoring specialization on alternative resources (Robinson & Wilson 1994; Skulason & Smith 1995). Likewise, in sympatry there are more opportunities for reinforcement of divergence if some of these same traits are also involved in sexual selection and mate choice (e.g. McKinnon et al. 2004). There is no risk of breeding with alternative forms in allopatry, but in sympatry this could result in reduced offspring fitness.

This study does show that adaptive divergence in Arctic charr tends to follow the same evolutionary lines in both allopatry and sympatry. The similarities between the polymorphic forms that result in each situation indicate niche availability, above all, plays the leading role in evolution of trophic specializations this species. The next chapter addresses the question of whether these trophic specializations are labile in contemporary time.

CHAPTER 3 – ECOLOGICALLY DRIVEN TROPHIC DIVERGENCE OVER CONTEMPORARY TIME SCALES

ABSTRACT

Selection on traits related to trophic ecology is thought to be an important contributing factor to adaptive divergence and speciation in many taxa. For several freshwater fish species, including Arctic charr, such selection is reflected in relationships between trophic ecology and phenotypic divergence. It is difficult, however, to examine the root causes of such divergence, as many of these populations have coexisted for thousand of years. Here, I address the hypothesis that selection on trophic related traits plays an important role in the beginning stages of divergence by comparing a recently translocated population with its source. Differences observed between the populations were analogous to those typical of trophic forms observed elsewhere, including divergence in head, body and gill raker morphology. I suggest these differences reflect contemporary shifts in trophic specialization in response to a human induced alteration of the lake community structure experienced by these charr. The results reported here support the idea that trophic ecology plays an important role in initiating divergence, and potentially, speciation.

INTRODUCTION

Divergence in characters related to trophic ecology has been noted in a wide variety of taxa, from birds, including the Galapagoes finches (*Geospiza* spp) (Grant & Grant 2002) and the African seedcracker (*Pyrenestes ostrinus*) (Smith 1987), to amphibians such as the tiger salamander (*Ambystoma tigrinum*) (Collins et al. 1993), to

freshwater fishes, especially pumpkinseed sunfish (Robinson et al. 1993), threespine stickleback (Schluter 1995), lake whitefish (Lindsey 1981), and Arctic charr (Jonsson & Jonsson 2001). The correlation between trophic ecology and certain traits suggests that selection driven by resource use plays a major role in adaptive divergence, and potentially speciation. However, it is difficult to directly examine the tempo and mode of the initial stages of this process in populations that have already diverged over thousands of years.

Arctic charr represent a classic study system for trophic specialization (Jonsson & Jonsson 2001). Much of the research done in this species has explored polymorphisms in sympatric populations (e.g. Saundland et al. 1992; Adams et al. 1998; Alekseyev 2002), but recent work demonstrates allopatric populations can also show substantial trophic specialization (see chapter 2). In either case, the results of trophic mediated selection pressures are manifest as variation in morphological, life history, and behavioral characters among forms (Skulason & Smith 1995). The patterns of divergence for these traits are strongly correlated with the size and hardness of a preferred prey type, as well as the limnological region in a lake where that particular prey is found (Jonsson & Jonsson 2001). The variety of trophic specializations that developed in this species over postglacial time scales has impressed evolutionary ecologists for some time, but I hypothesize it may not have taken thousands of years for some of these differences to develop.

A growing body of literature suggests measurable evolutionary change can arise in populations over humanly observable time scales (Hendry and Kinnison 1999; Kinnison and Hendry 2001). Likewise, extensive literature on phenotypic plasticity and

reaction norms demonstrates certain genotypes can produce a range of potentially adaptive phenotypes in response to altered environmental conditions (Robinson & Parsons; e.g. Trussell & Etter 2001; Conover 2003). In combination, these bodies of evidence suggest trophic divergence may arise over very short time scales in the wild. Indeed, trophic specializations evolve in Galapagos finches (Grant & Grant 1995) and phytophagous insects (Carroll et al. 1997) over just a few generations. However, contemporary trophic evolution has not been previously reported for wild fish populations.

Though the histories of most indigenous populations are unknown, there are a few opportunities for studying the initial stages of adaptive divergence in the wild. Human perturbations of ecological community structure, such as the introduction of exotic species, can serve as semi-natural experiments for directly examining evolutionary processes as they unfold (e.g. Carroll 1997; Stockwell and Weeks 1999; Streelman 2004). When records are sufficient to indicate sources and times of introductions, translocated populations sharing a common ancestry can be compared with each other or back to their ancestral source (e.g. Kinnison et al. 2001). This situation provides the opportunity to not only examine differences that develop between groups, but also the tempo and mode by which these specializations arise (Kinnison and Hendry 2001). Such information could shed some light onto the earliest processes involved in population divergence and speciation that must otherwise be inferred from long divergent populations.

Among freshwater fishes, translocations and introductions are common occurrences (Lever 1996), but a relatively small number of these have been well

documented. However, recent translocations of Arctic charr in the state of Maine are very well documented (Kircheis 1989). These actions were the result of conservation efforts aimed at preserving the endemic "Sunapee" form of Arctic charr thought to be found only in Floods Pond, Maine. The Maine Department of Inland Fisheries and Wildlife initially transplanted charr from this population into seven other systems throughout Maine. Of these, naturally reproducing Arctic charr persist in only two systems; Long Pond and Enchanted Pond (Frost 2001). Although this effort was made to preserve the particular characteristics of the "Sunapee" form, it is likely these translocated populations were subject to different environmental conditions in their new habitats, providing the impetus for potential change to their trophic specializations.

OBJECTIVES

This research considers the rate at which trophic specializations may change in the wild. The specific goals of this study are to determine 1) if trait divergence can be detected between the translocated population of Arctic charr in Long Pond and its ancestral source in Floods Pond; 2) if divergence observed in phenotype can be correlated with trophic ecology; and 3) whether the patterns of divergence observed here are similar to those described elsewhere for populations that diverged over much longer time periods.

METHODS

Fish Collection and Processing

Arctic charr included in this study were sampled from Floods Pond and Long Pond during both the summer and fall (table 3.1). Fish collection was attempted at Enchanted Pond, but only 5 individuals were obtained, so this population was not included in the analyses. During summer, fish were caught using monofilament gillnets (mesh size ½"-1" stretch), each set at a depth of approximately 30m for 12-24 hours. Charr obtained during the fall spawning season were captured using an Oneida style trapnet set over the primary spawning shoal in Floods Pond, and at several locations along the shoreline in Long Pond.

Live-caught individuals (fall samples) were anesthetized using MS-222 for processing, and all mortally sampled fish (summer samples) were placed on ice immediately after capture and photographed within 12 hours. Digital photographs were taken of the left side of every fish using a camera set at a fixed focal length, with a ruler included in each picture for size reference. Measurements that could not be obtained from these photographs, including head width (measured as interorbital distance), head depth (measured at the posterior edge of the skull), and weight, were also recorded at this time. Sex was determined for spawning individuals based on body shape and the presence of an everted ovipositor in females, or expression of milt in males. Live-caught fish were returned to the water after processing, and mortally sampled individuals were frozen to preserve them until they could be dissected.

During dissection, gill arches, sagittal otoliths, and stomach contents were removed from each fish. Sex and maturity status were also assessed at this time by

examining gonadal development. Morphological analyses presented here use only mature females caught during summer to avoid confounding trophic related divergence with disparate sex ratios and secondary sexual trait development.

Table 3.1. Sample locations with abbreviations, primary drainage, lake area and depth, and sampling dates.

Lake	Abb.	Area (hectares)	Mean (max) Depth (m)	Oxygen (mg/L)	Average Secchi (m)	Sampling Dates
Long Pond	LNP	107	12 (35)	10 @ 27m	8.3	7/2003 10/2003 10/2004
Floods Pond	FLP	257	12 (45)	7 @ 39m	7.3	10/2002 8/2003 10/2003 7/2004

Stocking History & Lake Characteristics

Approximately 100 mature Arctic charr (roughly 50 males and 50 females) were translocated from Floods Pond to Long Pond during the fall of 1977, and again in 1979. In each case, fish were transported by plane and stocked in an area of the lake that appeared to be suitable spawning habitat.

Information on the morphometry, limnology, and community composition of both lakes was obtained from the Public Educational Access to Environmental Information (PEARL) database, and by direct assessment during field sampling. Overall, both lakes are highly oligotrophic and relatively deep for lakes in Maine (table 3.1). They also exhibit saturated oxygen profiles throughout the entire depth of the lake during summer months. In addition to Arctic charr, Floods Pond supports populations of eastern brook charr, rainbow smelt, pumpkinseed sunfish, threespine stickleback,

ninespine stickleback (*Pungitius pungitius*), white sucker (*Catostomus commersoni*), and several other minnow species. Long Pond contains rainbow smelt, Arctic charr, a few minnows, and is stocked annually with eastern brook charr. Given these similarities, one might expect that charr in both lakes have access to a similar range of potential prey and face similar potential competitors. However, it is likely that the relative availability of different food items and pressure from potential competitors, such as smelt or brook charr, varies between the systems.

Shape Analysis

Relative warp scores were obtained from homologous landmarks placed on standardized digital images of each fish as described in chapter 2. The relative warp scores were then put into a discriminant functions analysis to test for and summarize the morphological divergence between populations. The resulting discriminant functions were regressed back onto the partial warps using tpsRegr, version 1.31 (Rohlf 2005) to aid interpretation.

Several linear measurements that could not be included in the relative warps analysis were also compared between populations. These were pectoral, dorsal, pelvic, anal, and caudal fin lengths, eye width, maxilla length, and body length (measured from anterior eye socket to posterior most point on the caudal peduncle), all derived from digital photos. One caliper measurement, interorbital distance, was also included as a metric for head width. All measurements were adjusted to a common body length of 185.00 mm using analysis of covariance. Principal components analysis was then used

to summarize the variation in these traits, and group differences were assessed using analysis of variance.

Rate of Divergence

The rate of change in body morphology occurring between the transplant population and its ancestral source was quantified in terms of haldanes, as described in Hendry & Kinnison (1999). This was calculated using the formula $h = [(x_2/s_p)-(x_1/s_p)]/g$. In this case, x_1 and x_2 are the mean discriminant function or principal component scores for each population, g is the estimated number of generations since the populations were separated, and s_p is the pooled standard deviation: $\sqrt{([SS_1+SS_2]/[(n_1-1) + (n_2 - 1)])}$. Since the actual generation time for these populations is unknown, I used an estimate of g=6.25 generations, which is expected to provide a conservative rate estimate as it is based on age of first maturation (age 4 for Floods Pond charr - Kircheis 1976).

Growth

Sagittal otoliths were examined to determine the age of individuals. To facilitate the reading of annuli under a dissecting scope, otoliths were placed whole in a small glass dish containing 50% glycerin solution and illuminated from the side using a fiber optic light source. Annuli were counted on both left and right otoliths (when available), and age was determined based on agreement between these counts. Mean size-at-age was compared between populations using analysis of covariance.

Population growth curves were also estimated using the Von Bertalanffy growth function (VBGF): $L_t = L_\infty(1-e^{-k(t-t0)})$ where L_t is the length at age t, L_∞ is the asymptotic length for the population, k is the Brody growth coefficient, and t0 is the age at which length = 0 (Ricker 1975). This model was fitted to the length at age data for each population using a combination of the best fit model approach and biological criteria, because there were few data points for very young fish. These growth curves were compared between populations using the analysis of residual sums of squares method described by Chen et al. (1992).

Gill Raker Morphology

The most anterior, left gill arch was extracted from each individual, and the gill filaments removed. These were placed in ethanol to prevent decay, and allowed to dry before being photographed. Individual arches were pressed flat between two sheets of plexiglass, and photographed along with a ruler for size reference using a digital camera mounted at a fixed focal length. From these images, measurements of gill raker height, width, and spacing of the first three rakers below the apex on the ventral side of the arch were obtained as described in chapter 2. The total number of gill rakers, mean gill raker height, width, and spacing, were compared between populations using analysis of variance.

Diet

Stomach contents removed from all mortally sampled individuals were examined under a dissecting scope to determine the presence of zooplankton, insect

larvae, and fish in their diet. The differences in stomach contents between populations were then assessed using a chi-squared test for each prey category.

Isotope Analysis

Analysis of carbon and nitrogen isotopes was conducted to further explore the trophic status of the recently transplanted population of Arctic charr in Long Pond with respect to its source, Floods Pond. Littoral and pelagic reference samples were obtained in each location during September 2005. Zooplankton were collected by multiple subsurface (1-2m) tows of a 250μm plankton net across each lake. The material collected from each tow was combined into one sample per location to account for spatial heterogeneity, and later sorted to remove any debris or algae present. Unioid mussels were collected to serve as a reference for the pelagic carbon signature, and were either gathered by snorkeling or using Ekman grabs in deeper water. Gastropods were used to approximate the littoral signature, and were removed from the substrate along the shoreline. Mollusk samples could not be obtained from Long Pond, so amphipod samples from Ekman grabs in this zone served as the littoral reference, while zooplankton was used for the pelagic reference. Arctic charr samples were comprised of dorsal muscle tissue removed just anterior of the dorsal fin. Both male and female Arctic charr were included in the analysis, and individuals from each population were specifically chosen to represent the range of age classes in each sample.

All samples were placed in a drying oven at 50°C for 48 hours. Charr samples were then ground using a mortar and pestle, while all other samples were left whole for shipment. All samples were placed in dry glass scintillation vials, which had been

soaked in deionized water for 24 hours, and capped with foil-lined lids. These were stored in a desiccator until they were shipped to the Environmental Isotope Laboratory at the University of Waterloo, Waterloo, Ontario, for analysis. Here, the remaining samples were ground to a fine homogenate powder using a Retsch MM301 (Retsch GmbH, Haan, Germany). Approximately 1mg of tissue was then used in the simultaneous analysis of stable carbon and nitrogen isotopes for each sample. All analyses were performed on a Micromass VG Isochrom continuous-flow isotope ratio mass spectrometer connected to a Carlo Erba elemental analyzer with an analytical precision of ± 0.2‰ for carbon and ± 0.3‰ for nitrogen determined by repeat analysis (n = 20) of the International Atomic Energy Agency standards CH6 for $\delta^{13}C$ and N1 and N2 for $\delta^{15}N$.

All stable isotope values are reported in conventional δ notation, where $\delta^{13}C$ or $\delta^{15}N = (R_{sample} - R_{standard})/R_{standard} \cdot 1000$ and $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$. The standards used for carbon and nitrogen analysis, respectively, were carbonate rock from the Peedee Belemnite formation (Craig 1957) and nitrogen gas in the atmosphere (Mariotti 1983). By convention, all international standard isotope values are set at 0‰.

Mean trophic position of charr in each lake was calculated using a two-end-member-mixing model to take into account omnivory in the diet: $\lambda + [\delta^{15}N_{charr} - (\delta^{15}N_{pelagic\ base} \bullet \alpha + \delta^{15}N_{littoral\ base} \bullet (1 - \alpha))] / 3.4$, where λ is the trophic position of the organism used to estimate the $\delta^{15}N$ base of each food chain; 3.4 is the $\delta^{15}N$ enrichment approximated for each trophic level (Post et al. 2000); and α is the proportion of carbon in an organism ultimately derived from the base of the pelagic food web: $\alpha = (\delta^{13}C_{charr} - \delta^{13}C_{littoral\ base}) / ((\delta^{13}C_{pelagic\ base} - \delta^{13}C_{littoral\ base})$ (Post et al. 2000). For Floods Pond,

mussels and snails were used to estimate the base nitrogen isotope signature for the pelagic and littoral food chains, respectively, while zooplankton and amphipods were used for Long Pond. The λ value assigned to all the organisms used to estimate the base of each food chain was 2. However, 3.4‰ was subtracted from the $\delta^{15}N$ of the pelagic base in Long Pond to account for expected trophic enrichment (e.g. Post 2002), and known differences in the mixed assemblage isotopic signature of zooplankton and filter feeding mussels found in Floods Pond. Correlations between α and forklength, as well as trophic position and forklength, were calculated to evaluate changes in diet with size (and age) of fish in each population.

RESULTS

Shape Analysis

Discriminant functions analysis revealed significant differences in body morphology between the transplant population and its ancestral source (Wilks $\Lambda = 0.2851$, approximate $F_{(32, 53)} = 4.152$, $p<0.001$). With only two populations included in the analysis, the single discriminant function revealed a gradient defined mainly by head depth and overall body depth (figure 3.1). Floods Pond charr cluster towards the deeper bodied, larger-headed end of the spectrum, while those from Long Pond appear to have a thinner, more streamlined, overall body shape. The reclassification rate of individuals from both groups also indicates strong divergence in form between populations (overall jackknifed classification rate = 85%).

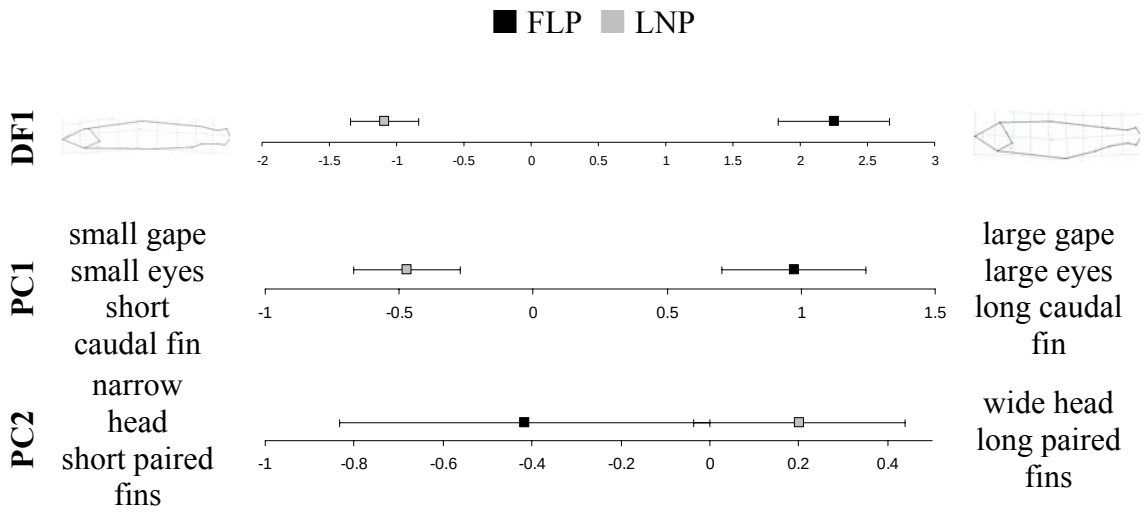

Figure 3.1. Mean discriminant function and principal component scores for each population (± 95% CI). Interpretations of high negative and positive scores are indicated at each end of the axis. Images show thin-plate spline transformations depicting body shapes at the extremes of the discriminant function. These visualizations have been exaggerated three-fold to aid interpretation.

There are also significant differences between populations for the linear measures included in this analysis of shape. These differences were best summarized by two varimax rotated principal components, which together explained 49% of the variation in the data set (table 3.2). The first principal component mainly explained variation in maxilla length, eye width, and caudal fin length. An ANOVA on PC1 revealed a significant difference between Long and Floods Pond

Table 3.2. Correlations between principal components and morphometric traits, and the percent of total variance explained by each component. Characters highly correlated with a principal component are noted with an asterisk.

	PC1	PC2
Head Width	-0.322	0.687*
Maxilla Length	0.608*	-0.055
Eye Width	0.784*	0.009
Anal Length	0.021	0.565*
Dorsal Length	-0.036	0.237
Pectoral Length	0.373	0.651*
Pelvic Length	-0.119	0.696*
U. Caudal Length	0.811*	-0.139
L. Caudal Length	0.838*	-0.040
Variance Explained	29%	20%

individuals along this gradient, with the translocated population having smaller eyes, shorter maxilla, and shorter caudal fins ($p<0.001$). Head width and paired fin lengths all loaded high on principal component two, which also significantly differentiates between populations ($p=0.007$), with Long Pond fish having somewhat wider heads and longer fins (figure 3.1).

Rate of Divergence

The rate of change in body morphology between Long and Floods Pond was estimated to be 0.534 haldanes using the mean discriminant function scores, 0.313 haldanes for PC1 (maxilla, eye, and caudal fin size) and -0.103 for PC2 (head width and paired fin lengths).

Size-at-age

The two populations also exhibited differences in mean size at age ($p<0.001$). This was supported by differences in the Von Bertalanffy growth function between groups ($F_{5,79} = 2.31$; $p=0.052$) (figure 3.2). Although Floods Pond had the larger asymptotic length value, it also had a smaller growth coefficient than Long Pond.

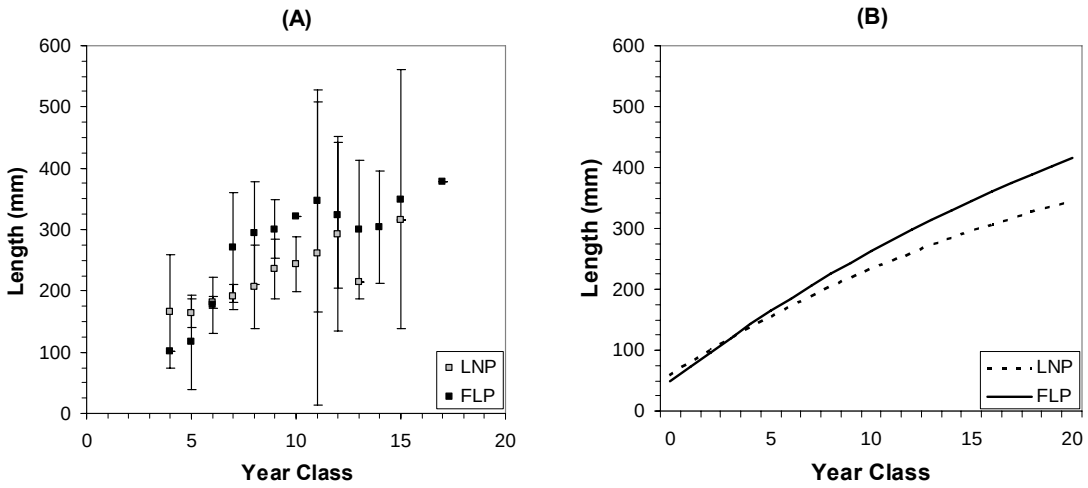

Figure 3.2. (A) Plot of mean size at age (± 95% CI) for individuals collected from Floods and Long Pond. (B) VBGF for both populations (note: k may be underestimated due to the lack of very young individuals in the data set).

Gill Raker Morphology

Two aspects of gill raker morphology, spacing and width, differed between populations (p=0.017 and p=0.013 respectively), but length (p=0.328) and number (p=0.247) did not. On average, Floods Pond charr have thicker, more widely spaced gill rakers than those from Long Pond (figure 3.3).

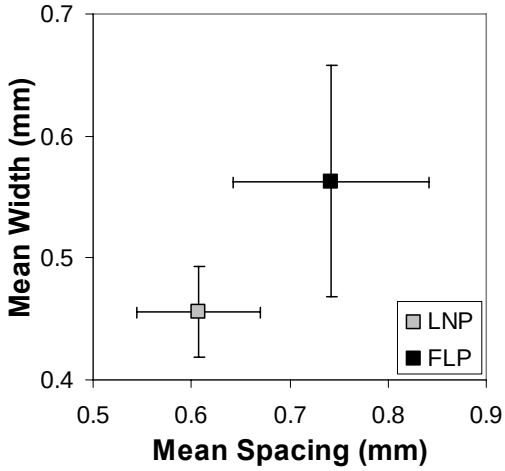

Figure 3.3. Mean gill raker width and spacing (± 95% CI).

Diet

Analysis of stomach contents revealed differences in the frequency with which prey items were consumed between populations. Although the majority of individuals from both populations included insect larvae, mainly diptera, in their diet, a larger proportion of individuals from Floods Pond also fed on fish (table 3.3).

Table 3.3. Percent individuals from each population with a given prey category present in its stomach contents. Numbers in parentheses represent the number of individuals from which stomach contents were obtained for each population. P-values were obtained from Pearson's chi-squared test.

	plankton	Insect	fish
FLP (38)	3%	76%	50%
LNP (91)	1%	90%	14%
P-value	**	0.006	< 0.001

** insufficient data for significance test

Isotope Analysis

The differences in diet observed for stomach contents are supported over longer time scales by analysis of carbon and nitrogen stable isotope ratios in the muscle tissue of these fish (figure 3.4). The average proportion of diet obtained from a pelagic source, α, for Long Pond was 0.272 (95% CI = ±0.018), while Floods Pond fish fed almost entirely on pelagic food sources (α = 0.953 ±0.015). Calculation of trophic level using the two-end-member-mixing model, indicated that charr from Floods Pond are also feeding at a significantly ($p<0.001$) higher average trophic position (4.701 ±0.046) than those from Long (4.057 ±0.088). This divergence in diet was consistent for all fish included in this analysis, as individuals from each population did not overlap at all in the proportion of diet obtained from the pelagic food web.

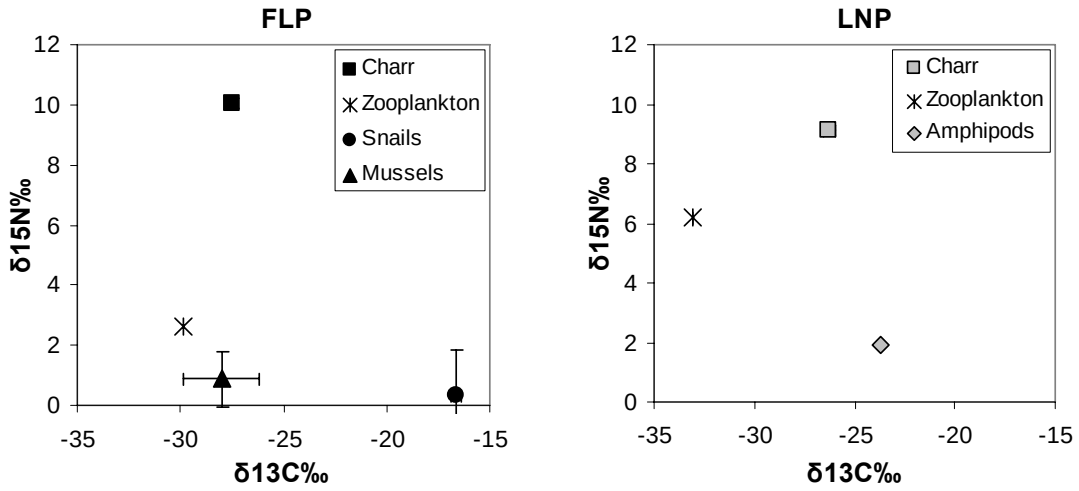

Figure 3.4. Isotope plots for Arctic charr and organisms representative of the respective pelagic and littoral food webs of each lake. Taxa means (± 95% CI) are reported for both stable isotopes (no error bars were calculated for the zooplankton and amphipod signatures from Long Pond because organisms were combined into one homogenous sample; error bars for charr samples are present, but very small).

Trends associated with α and trophic position with length indicate charr from Floods Pond feed on fish from a relatively small size, while those from Long undergo a clear ontogenic niche shift at larger sizes (figure 3.5a). The relationship between trophic position and length was analogous to that of trophic position and age. There is a strong positive correlation ($r=0.832$, $p<0.001$) between trophic position and length for individuals from Long Pond, but no significant trend associated with those from Floods ($r=0.113$, $p=0.9$). There is also a significant negative correlation between α and body size for Long Pond charr ($r=-0.433$, $p=0.076$), but not for those from Floods ($r=-0.582$, $p=0.003$) (figure 3.5b).

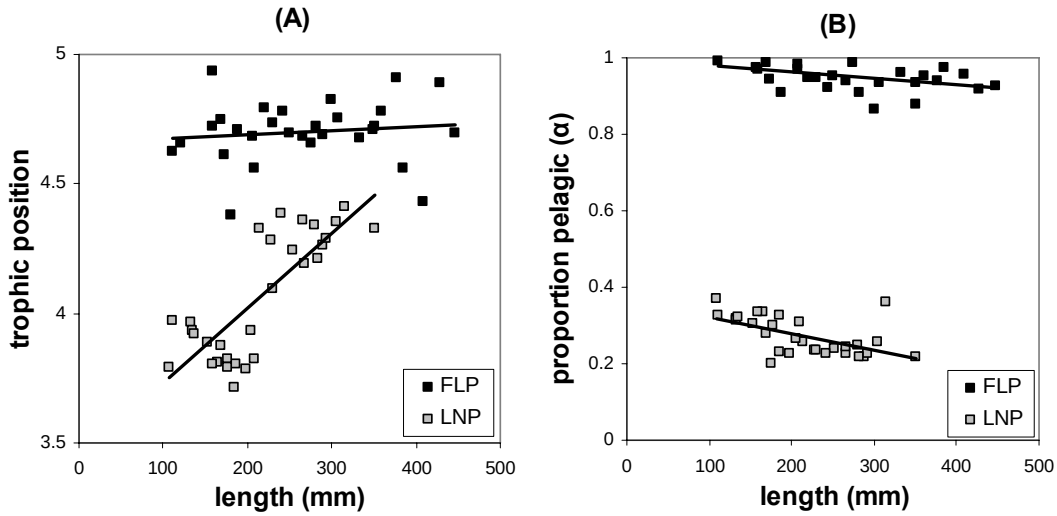

Figure 3.5. (A) Calculated trophic position vs. length for all individuals. (B) Plot of α (proportion of diet obtained from the pelagic food web) vs. length for all individuals analyzed.

DISCUSSION

The results presented here demonstrate significant divergence in charr phenotypes can develop over only a few generations. It also appears that these differences reflect a shift in trophic ecology experienced by the transplanted population, as evidenced by stomach contents, stable isotope signatures, and (as will be discussed) some correspondence between observed trait divergence and known patterns of trophic specialization in this species. Overall, these results support the hypothesis that trophic specializations in Arctic charr, which typify population divergence or even incipient speciation, can change over very short time scales in response to likely changes in niche space.

Although some individuals in Long Pond do consume fish as part of their diet, there is clearly a much smaller percentage of the population that does so when compared to Floods Pond charr. Only 14% of the charr sampled from Long Pond with

food in their stomachs consumed fish, compared to 50% of charr from Floods Pond. Trends found in this small snapshot of the diet of these individuals are consistent with those associated with the stable isotope signatures of the populations. Long Pond charr appear to derive a much greater proportion of the ^{13}C in their muscle tissue from the littoral food web, and they feed at a lower trophic position than charr from Floods Pond. This is exactly what one would expect for fish that prey predominantly on littoral insect larvae and pupae and less extensively on pelagic fish like smelt.

The differences in morphology observed for Arctic charr in Long Pond show a clear trend of divergence away from the trophic related features of their ancestors. As described in a previous study of trophic specializations among indigenous Arctic charr populations in Maine, Floods Pond charr represent a clear piscivorous specialist (see Chapter 2). Comparatively, the morphology of charr from Long Pond indicates they may be diverging more toward traits consistent with a form that picks smaller prey items such as migrating insect larvae and pupae from the water column. Shape analysis shows these fish have thinner bodies overall and shorter caudal fins. This is consistent with a pelagic form, but still more streamlined and thus better suited for cruising in the water column than the larger piscivores (Webb 1975). Individuals from the translocated population also tend to have smaller heads with shorter maxilla, which is indicative of a smaller gape size. The gill rakers of fish in this population also tend to be thinner and more closely spaced than those of fish from the ancestral population. The changes in these features are all associated with Arctic charr forms that utilize smaller prey items (Jonsson & Jonsson 2001; Alekseyev 2002).

Charr from Long Pond also appear to grow faster early in life, but reach a smaller asymptotic size than charr from Floods. This may be a consequence either of feeding on a food source that does not put as much of a premium on size to be an effective predator, or one that does not provide as much nutrition per prey item (Wootton 1998). One might presume a correlation between diet and asymptotic size is reflective of growth advantages associated with shifting to a more energy rich prey item like fish, or the requirement that piscivores be relatively large in order to capture and consume these larger food items (e.g. Snorrason et al. 1994). Both populations of charr show a relationship between fish size and age and diet, however they differ in their ontogenic patterns. Trends associated with proportion of diet obtained from the pelagic food web (α) and trophic position with length indicate charr from Floods Pond feed on fish from at a relatively small size, while those from Long undergo a clear ontogenic niche shift to feeding at a higher trophic level at larger sizes (figure 3.5a). This is evident by a strong positive correlation ($r=0.832$) between trophic position and length for individuals from Long Pond, but only a very slight positive trend associated with those from Floods ($r=0.113$) since even the youngest charr sampled were already feeding on fish. Although fish from Long Pond shift to feeding at a higher trophic level, α calculations indicate they are not doing so by including planktivorous fish in their diet, because they are including a higher percentage of their diet from the littoral food web at this stage.

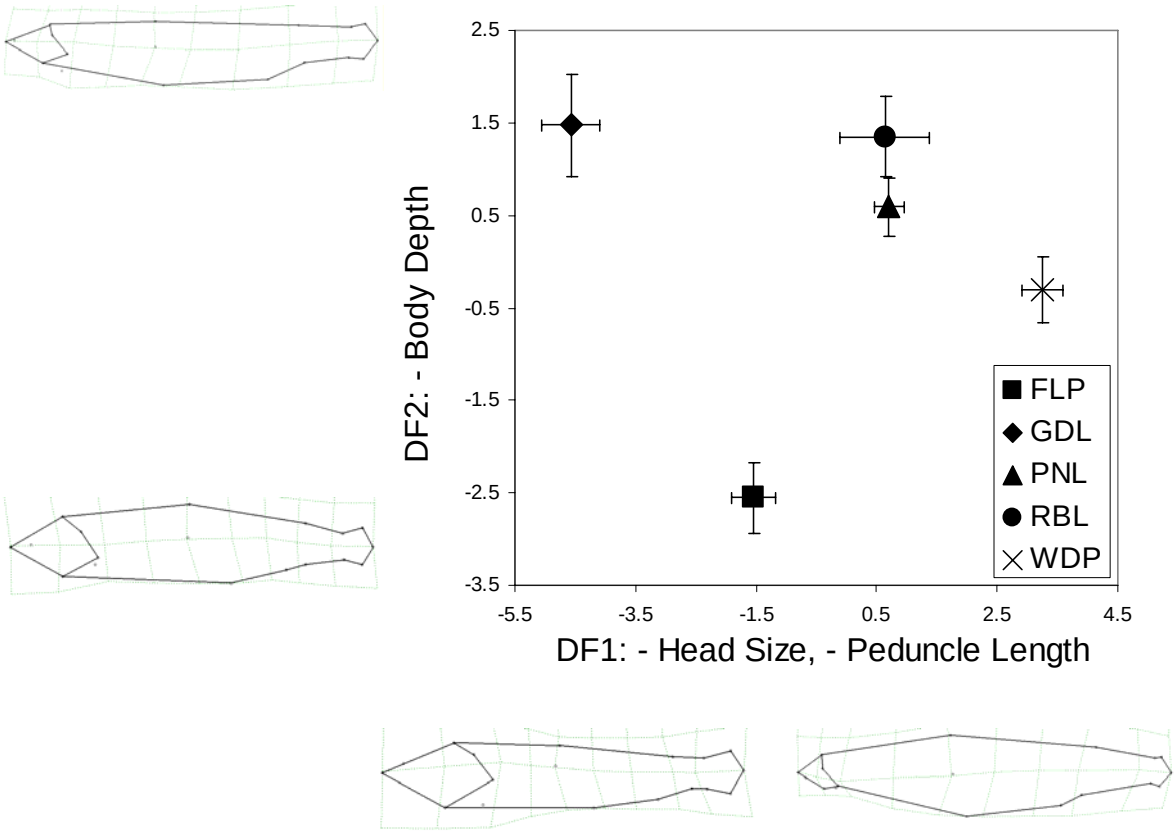

Figure 3.6. Shape variation among indigenous and translocated populations of Arctic charr in Maine. Plot shows mean discriminant function scores for each population with 95% confidence intervals. Axis labels indicate features strongly associated with each discriminant function, and the direction of this relationship is indicated by a "+" or "-" sign. Transformation grids show extremes of the shape variation associated with each function. These visualizations have been exaggerated three-fold to aid interpretation. (FLP=Floods Pond, GDL=Gardner Lake, PNL=Penobscot Lake, RBL=Rainbow Lake, WDP=Wadleigh Pond, and LNP=Long Pond).

In combination, the trends in divergence observed for diet, body shape, gill raker design, and life history of these populations suggest a broad spectrum shift in trophic specialization. However, the population of charr in Long Pond exhibits a mosaic of specialization traits. The mean head size of these fish is not smaller in all dimensions when compared to Floods Pond, as one would anticipate for a pelagic feeding insectivore (Jonsson & Jonsson 2001; Chapter 2). Likewise, their average fin size is a

bit larger than Floods Pond fish, rather than the smaller size observed for another insectivorous population in Maine (see Chapter 2). This would provide more maneuverability, and may indicate these fish are spending more time in the littoral zone. It should also be noted that these populations, while clearly divergent, are still more similar to each other in many traits than either is to any of the indigenous populations in Maine examined in chapter 2 (figure 3.6). Nonetheless, the calculated rates of morphological divergence between these populations in haldanes for traits like body shape, fin lengths, gape and eye size (from DF and PC1&2 results), indicate not only that these rates are comparable to some of the fastest rates of phenotypic change estimated for other organisms over comparable time scales (see Kinnison & Hendry 2001). By that standard, contemporary trait changes observed in Maine charr can justifiably be considered "rapid" (Hendry & Kinnison 1999).

It is perhaps surprising that such a shift in specialization occurred despite the availability of similar prey items in both lakes. Highly specialized piscivorous charr from Floods Pond could have continued to feed primarily on smelt in Long Pond, but this does not appear to have been the case. Hence, simply the availability of a forage item is not sufficient to fully retain this specialization. Rather, it appears that a more complicated interaction exists with the relative benefits of utilizing a particular food resource in a given lake system. Charr introduced to Long Pond may have encountered an especially abundant and high quality invertebrate resource and relatively fewer forage fish, or a larger population of piscivorous competitors in the more abundant brook trout population in this lake. In addition, the smelt population may have declined in response to the increased predation pressure. These factors could work alone or in

synergy to favor a diet with more reliance on insect larvae and pupae. Likewise, there is no reason to presume piscivory is a universally superior diet strategy. Instead, it is feasible that such a specialization in Floods Pond reflects necessity rather than opportunity. I do not have direct estimates of relative insect abundance, but the fact that Floods Pond charr may grow more slowly than those from Long Pond during their early years, when insects would comprise the bulk of their diets, provides indirect evidence that this may not be a particularly abundant food source. A switch to piscivory might therefore be critical for fish to attain a suitable size for reproduction in Floods Pond, but may be less important for charr in Long Pond. In a sense, a paucity of alternative food resources in Floods Pond may be more important to maintaining its piscivorous charr form than the abundance of smelt.

The apparent association between the phenotypic differences detected and trophic ecology suggests the divergence detected here is indeed adaptive and not solely the result of founder effects or drift. It is also likely that some component of contemporary trait divergence is due to phenotypic plasticity. Recent diet and habitat manipulation studies in Arctic charr have evidenced a degree of plasticity in similar traits (Adams et al. 2003, Peres-Neto & Magnan 2004; Andersson et al. 2003, 2005), but this study was not designed to distinguish the relative contributions of these two mechanisms. Nonetheless, it seems reasonable to suggest the process of divergence examined here is analogous to that which led to trophic specializations in other allopatric and sympatric populations of charr. If so, then considerable incipient steps toward such long term divergence can indeed arise very quickly in populations subject to shifts in relative community structure.

It also appears unlikely that specific trophic specializations can be maintained outside of the complex set of habitat conditions that foster them. Clearly, this has implications for the use of translocations in the preservation of endemic specialists. I will consider such conservation implications for Maine charr and other organisms in my final chapter.

CHAPTER 4 - MANAGEMENT IMPLICATIONS

The objectives presented in the current Arctic charr management plan for the state of Maine (Frost 2001) include maintaining the 14 self-sustaining populations of Arctic charr in the state, as well as maintaining the genetic integrity of these populations. However, due primarily to lack of funding and support, little is known about the general ecology of these populations, making it difficult to make informed management decisions. I am therefore hopeful the work I presented in this thesis will provide useful insights into conserving Maine's Arctic charr. In particular, I believe my research can provide insight into habitat requirements and uniqueness of these populations, as well as information that may be useful in developing conservation strategies.

Learning more about the general ecology and distinctive features of Arctic charr in Maine is becoming increasingly important as populations are being threatened by changing environmental conditions and human perturbations to their ecosystems. In fact, there are indications that at least two populations are currently at risk of local extinction. A month of efforts to capture charr in Big Reed Pond by the Department of Inland Fisheries and Wildlife during the fall of 2005, recovered only a single fish. Our own efforts to trap charr in Green Lake during the same period were also unsuccessful. These failed collection attempts are worrisome, particularly for the population in Green Lake, which has been described as unique among charr in Maine (a very small form that lives at extreme depths) (Kircheis 1985).

DIET AND HABITAT

According to the current Arctic charr management plan, the state describes bluebacks as primarily planktivorous at all ages, but recognizes they may supplement their diet with other prey items (Frost 2001). The results of this research, however, suggest bluebacks in Maine are not primarily planktivorous as adults and vary widely in the items they incorporate into their diets. Although zooplankton was found among stomach contents of individuals in each lake, a much higher proportion included insect larvae and pupae. This pattern could be an artifact of sampling populations in mid-summer, although insect larvae were still found in a majority of fish sampled during late winter in Rainbow Lake. It is also interesting to note the large proportion of individuals that included larger prey items in their diets, as well as the variation in the type of prey each population fed on most heavily. Charr from Floods Pond, Penobscot Lake, and Long Pond, all included fish in their diets. While a large proportion of individuals from Gardner Lake and Rainbow Lake included benthic organisms such as amphipods and mollusks. Ultimately, a more varied diet makes sense given that the lakes containing charr in Maine tend to be very oligotrophic and probably produce a relatively low biomass of zooplankton.

Results of the stable isotope analysis conducted on tissue samples from Floods, Long, and Wadleigh Pond provide long term support for trends seen in stomach contents. Charr from Floods Pond do obtain a very high proportion of their diet from the pelagic food web (0.95 ± 0.01), and the relative amount of nitrogen-15 in their tissues suggests they feed at an average trophic level of 4.7 (± 0.05). This suggests Arctic charr are a top predator in this system. In Long Pond, it is again very unlikely

these fish rely on plankton as a primary food source as adults because these fish obtain only a small proportion of their diet from the pelagic food web (0.27 ± 0.02). Instead, it appears that charr in Long Pond rely more heavily on the littoral food web, most likely diptera larvae. Arctic charr in Wadleigh Pond are probably more reliant on zooplankton as a food source than any of the other populations examined in this study. Results of the stable isotope analysis suggest they obtain a significant portion of their diet (0.68 ± 0.14) from the pelagic food web, while feeding at a lower trophic level than noted for the other two populations (3.26 ± 0.11). However, there is a strong correlation between age and trophic level ($r = 0.73$), as well as a trend towards a decreasing proportion of food coming from pelagic sources as fish from Wadleigh Pond age.

Uniqueness of Charr within Maine

As noted in my introductory chapter, Maine's charr represent the only indigenous populations of this species in the United States outside of Alaska. It should also be noted that Arctic charr in Maine and Alaska represent very divergent lineages, with Maine charr being part of the putative Acadian lineage identified by Brunner et al. (2001) that also includes populations of charr in Quebec and New Brunswick. Among the five global lineages of Arctic charr identified by this study, this Acadian group is by far the most restricted in its distribution. Being part of this distinctive clade, which also includes the southernmost indigenous populations of charr in North America, should convey some unique status that warrants conservation.

Work with mitochondrial DNA (Kornfield & Kircheis 1994) and microsatellite variation (Bernatchez et al. 2002) suggested Maine charr have nearly all been isolated

from one another for thousands of years. At the same time, this genetic work provided no evidence that the blueback and Sunapee forms of charr represent separate, monophyletic lineages. This finding eliminated the separate subspecies status of these forms, and as a consequence interest in the special nature of the Floods Pond charr declined. My work on the phenotypic specializations of these forms suggests that abandonment of the unique status of this population was perhaps premature.

Arctic charr in Maine show considerable evidence of trophic specializations developed over postglacial time. While these specializations may not warrant subspecies status for these populations, they do represent important variation within the species. Diet differences among these populations are reflected by the broad spectrum of variation in morphological and life history features. The traits showing most divergence among these groups include head size, body depth, gape size, fin lengths, gill raker morphology, and size at age; all characters strongly correlated with trophic ecology in this species (Jonsson & Jonsson 2001). It is almost certain many of these same features provided early taxonomists and anglers with a perception of divergence between the blueback and Sunapee form; my work suggests considerable diversity also exists among these putative blueback populations.

CONSERVATION STRATEGIES

My work indicates the diversity in form seen among Arctic charr in Maine can be attributed to differences in their diet and habitat use. Though introductions of salmonid predators (e.g., lake charr, *Salvelinus namaycush* or landlocked Atlantic salmon, *Salmo salar*) and changes in lake water quality (e.g. eutrophication and loss of

oxygen in deep water refuges) pose obvious direct threats to charr populations, conservation of Maine's specialized charr forms may also require preservation of the trophic communities and habitats that support those specializations. Management actions that threaten community structure, such as introductions of competitor species or extinctions of forage species, may lead to local charr extinctions. Certainly, loss of smelt from Floods Pond would be disastrous for conserving the Sunapee form, but at the same time stocking of smelt into other lakes may be just as damaging. For example, competition by introduced smelt may have been a factor in the extinction of bluebacks in the Rangeley Lakes system in the early 1900s. Likewise, declines in charr in Big Reed Pond have coincided with establishment of an illegally introduced smelt population.

My results also suggest that conservation of Maine's charr forms requires consideration of more than just extinction effects. Specializations found among Maine charr populations may be unstable in response to different trophic opportunities, even in contemporary time. Long Pond charr already exhibit changes in some of the features that typify the Sunapee form a mere twenty-five years following translocation. Interestingly, these changes have occurred in a system where similar forage species are available to those in Floods Pond. This suggests that the specializations of Maine's charr are dependent on complex interactions that shape the relative costs and benefits of utilizing different diet items in different lake systems. Ultimately, Floods Pond may be the only lake in the state capable of maintaining the specialized Sunapee form that is such an important part of Maine's fish fauna. The same may be true of the

benthivorous specialists in Gardner Lake, or the small pelagic form found in Wadleigh Pond.

This is not to say that translocated populations cannot thrive. The Arctic charr populations in Long and Enchanted Ponds are testimony enough to show that persistence is possible in a new lake system (although they do represent only two successes out of 11 attempts). However, these newly established populations should probably be regarded as their own evolutionary entities (Stockwell et al. 2003). In this respect, translocations may serve a role in preserving the evolutionary process and genetic lineage of the species, if not its full diversity. On the other hand, divergence between translocated and source populations may limit the value of translocated populations for use in recovery or supplementation of a collapsed source (e.g. Stockwell & Weeks 1999).

Although the Arctic charr faces many threats to its persistence in Maine, one of the biggest may simply be lack of information. Even with the scope of data presented here, there are many unknowns concerning the general ecology and life history of most Arctic charr populations in Maine. For example, the spawning sites of these fish are a mystery in all lakes other than Floods Pond. With a sample of just six populations I uncovered an unexpectedly wide range of trait variation and diet specialization, but the remaining eight populations have yet to be examined. Characterizing all of Maine's charr populations should be a research priority, given that additional populations may contain other specialized forms that would merit their own management considerations.

BIBLIOGRAPHY

Adams, C.E., Fraser, D., Huntingford, F.A., Greer, R.B., Askew, C.M. and Walker, A.F. 1998. Trophic polymorphism amongst Arctic charr from Loch Rannoch, Scotland. Journal of Fish Biology, 52:1259-1271.

Adams, C.E., Fraser, D., McCarthy, I., Shields, S., Waldron, S. and Alexander, G. 2003. Stable isotope analysis reveals ecological segregation in a bimodal size polymorphism in Arctic charr from Loch Tay, Scotland. Journal of Fish Biology, 52:1259-1271.

Adams, C.E., Woltering, C. and Alexander, G. 2003. Epigenetic regulation of trophic morphology through feeding behavior in Arctic charr, *Salvelinus alpinus*. Biological Journal of the Linnean Society, 78:43-49.

Alekseyev, S.S., Samusenok, V.P., Matveev, A.N., and Pichugin, M.Y. 2002. Diversification, sympatric speciation, and trophic polymorphism of Arctic charr, *Salvelinus alpinus* complex, in Transbaikalia. Environmental Biology of Fishes, 64:97-114.

Andersson, J. 2003. Effects of diet-induced resource polymorphism on performance in arctic charr (*Salvelinus alpinus*). Evolutionary Ecology Research, 5:213-228.

Andersson, J., Bystrom, B., Persson, L. and De Roos, A.M. 2005. Plastic resource polymorphism: effects of resource availability on Arctic char (*Salvelinus alpinus*) morphology. Biological Journal of the Linnean Society, 85:341-351.

Bean, T.H. 1887. Description of a supposed new species of charr (*Salvelinus aureolus*), from Sunapee Lake, New Hampshire. Proceedings of the US National Museum, 10:628.

Bernatchez, L., and Dodson, J.J. 1990. Allopatric origin of sympatric populations of Lake whitefish (*Coregonus clupeaformis*) as revealed by mitochondrial-DNA restriction analysis. Evolution, 44:1263-1271.

Bernatchez, L., Rhudderch J.G., and Kircheis F.W. 2002. Microsatellite gene diversity analysis in landlocked Arctic char from Maine. Transactions of the American Fisheries Society, 131:1106-1118.

Bodaly, R.A. 1979. Morphological and ecological divergence within the lake whitefish (*Coregonus clupeaformis*) species complex in Yukon Territory.

Brunner, P.C., Douglas, M.R., Osinov, A., Wilson, C.C., and Bernatchez, L. 2001. Holarctic phylogeography of Arctic charr (*Salvelinus alpinus* L.) inferred from mitochondrial DNA sequences. Evolution, 55:573-586.

Carroll, S.P., Dingle, H. and Klassen, S.P. 1997. Genetic differentiation of fitness-associated traits among rapidly evolving populations of the soapberry bug. Evolution, 51:1182-1188.

Chen, Y., Jackson D.A., and Harvey H.H. 1992. A comparison of von Bertalanffy and polynomial functions in modeling fish growth data. Canadian Journal of Fisheries and Aquatic Sciences, 49:1228-1235.

Collins, J.P., Zerba, K.E., and Sredl, M.J. 1993. Shaping intraspecific variation – development, ecology and the evolution of morphology and life-history variation in tiger salamanders. Genetica, 89:167-183.

Conover, D.O. and Present, T.M.C. 1990. Countergradient variation in growth rate: compensation for length of the growing season among Atlantic silversides from different latitudes. Oecologica, 83:316-324.

Craig, H. 1957. Isotopic standards for carbon and oxygen and correction factors for mass spectrometric analysis of carbon dioxide. Geochim. Cosmochim. Acta, 12: 133-149.

Dobzhansky, T. 1946. Complete reproductive isolation between two morphologically similar species of *Drosophila*. Ecology, 27:205-211.

Everhart, W.H. 1950. The blueback trout, *Salvelinus oquossa* (Girard), in Maine. Copeia, 3:242.

Fraser, D., Adams, C.E. and Huntingford, F.A. 1998. Trophic polymorphism among Arctic charr *Salvelinus alpinus* L., from Loch Ericht, Scotland. Ecology of Freshwater Fish, 7:184-191.

Frost, F.O. 2001. Arctic charr management plan. Maine Department of Inland Fisheries and Wildlife report, 20pp.

Girard, C. 1854. *Salmo oquassa* Girard. Proceedings of the Boston Society of Natural History, 4:262-263.

Grant, P.R. 1986. Ecology and Evolution of Darwin's Finches. Princeton University Press, Princeton, New Jersey. 488p.

Grant, P.R. and Grant. B.R. 1995. Predicting microevolutionary responses to directional selection on heritable variation. Evolution, 49:2411-251.

Grant, P.R. and Grant. B.R. 2002. Unpredictable evolution in a 30-year study of Darwin's finches. Science, 296:707-711.

Greenwood, P.H. 1984. African cichlids and evolutionary theories. Evolution of fish species flocks, A.A. Echelle and I. Kornfield eds. University of Maine at Orono Press, Orono, Maine. p141-154.

Hendry, A.P. and Kinnison, M.T. 1999. The pace of modern life: measuring rates of contemporary microevolution. Evolution, 53:1637-1653.

Hendry, A.P., Wenburg, J.K., Bentzen, P., Volk, E.C. and Quinn, T.P. 2000. Rapid evolution of reproductive isolation in the wild: evidence from introduced salmon. Science, 290:516-518.

Jonsson, B. and Jonsson N. 2001. Polymorphism and speciation in Arctic charr. Journal of Fish Biology, 58:605-638.

Kendall, W.C. 1914. The fishes of New England. The salmon family, Part I: The trout or charrs. Memoirs of the Boston Society of Natural History. 8:1-103.

Kinnison, M.T. and Hendry, A.P. 2001. The pace of modern life II: from rates of contemporary microevolution to pattern and process. Genetica, 112-113:145-164.

Kinnison, M.T., Unwin, M.J., Hendry, A.P. and Quinn, T.P. 2001. Migratory costs and the evolution of egg size and number in introduced and indigenous salmon populations. Evolution, 55:1656-1667.

Kircheis, F.W. 1976. Reproductive Biology and Early Life History of the Sunapee Trout of Floods Pond, Maine. Transactions of the American Fisheries Society, 105:615-619

Kircheis, F.W. 1980. The landlocked charrs of Maine: the Sunapee and the blueback. in Charrs, salmonid fishes of the genus *Salvelinus*. E.K. Balon ed. DR.W. Junk Publishers, The Hague, The Netherlands. p. 749-755.

Kircheis, F.W. 1985. Sport fishery management of a lake containing lake trout and an unusual dwarf Arctic char. Fisheries, 10:6-8.

Kircheis, F.W. 1989. History of landlocked Arctic charr management in Maine, USA. Physiol. Ecol. Japan, Spec. Vol 1: 615-623.

Kornfield, I. and Kircheis, F. 1994. Mitochondrial DNA and conservation of an aboriginal Arctic char (*Salvelinus alpinus oquassa*) from Floods Pond, Maine. Canadian Journal of Fisheries and Aquatic Sciences, 51:62-67.

Kornfield, I., K.F. Beland, J.R. Moring, and F.W. Kircheis. 1980. Genetic similarity among endemic Arctic char (Salvelinus alpinus) and implications for their management. Canadian Journal of Fisheries and Aquatic Sciences, 38:32-39.

Lauder, G.V. 1983. Functional and morphological bases of trophic specialization in sunfishes (Teleostei, Centrarchidae). Journal of Morphology, 178:1-21.

Lavin, P.A. and McPhail J.D. 1986. Adaptive divergence of trophic phenotype among fresh-water populations of the threespine stickleback (*Gasterosteus aculeatus*). Canadian Journal of Fisheries and Aquatic Sciences, 43:2455-2463

Lever, C. 1996. Naturalized fishes of the world. Academic Press, San Diego CA.

Lindsey, C.C. 1981. Stocks are chameleons: plasticity in gill rakers of coregonid fishes. Canadian Journal of Fisheries and Aquatic Sciences, 38:1497-1506.

Magnuson, J.J. and Heitz, J.G. 1971. Gill raker apparatus and food selectivity among mackerels, tunas, and dolphins. Fisheries Bulletin, 69:361-370.

Mariotti, A. 1983. Atmospheric nitrogen is a reliable standard for natural ^{15}N abundance measurements. Nature, 303:685-687.

Mayr, E. 1942. Systematics and the Origin of Species. Columbia University Press. 334p.

McKinnon, J.S., Mori, S., Blackman, B.K., David, L., Kingsley, D.M., Jamieson, L., Chou, J. and Schluter, D. 2004. Evidence for ecology's role in speciation. Nature, 429:294-298

Meyer, A. 1987. Cost of morphological specialization: feeding performance of the two morphs in the trophically polymorphic cichlid fish, *Cichlasoma citrinellum.* Oecologia, 80:431-436.

PEARL. The source for environmental information on Maine. Senator George J. Mitchell Center for Environmental and Watershed Research. http://www.pearl.maine.edu/default.htm

Peres-Neto, P.R. and Magnan, P. 2004. The influence of swimming demand on phenotypic plasticity and morphological integration: a comparison of two polymorphic charr species. Oecologia, 140:36-45.

Post, D.M., M.L. Pace, and N.G. Hairston Jr. 2000. Ecosystem size determines food-chain lengths in lakes. Nature, 405:1047-1049.

Rasband, W. 2004. ImageJ Version 1.32j. National Institutes of Health.

Reznick, D.N., Shaw, F.H., Rodd, F.H. and Shaw, R.G. 1997. Evaluation of the rate of evolution in natural populations of guppies (*Poecilia reticulata*). Science, 275:1934-1937.

Ricker, W.E. 1975. Computation and interpretation of biological statistics of fish populations. Bulletin of the Fisheries Research Board of Canada, 191:382 p.

Robinson, B.W., Wilson D.S., Margosian A.S., and Lotito P.T. 1993. Ecological and morphological differentiation of pumpkinseed sunfish in lakes without bluegill sunfish. Evolutionary Ecology, 7:451-464.

Robinson, B.W. and Wilson, D.S. 1994. Character release and displacement in fishes: a neglected literature. American Naturalist 144:596-627.

Robinson, B.W. and Parsons, K.J. 2002. Changing times, spaces, and faces: tests and implications of adaptive morphological plasticity in the fishes of northern postglacial lakes. Canadian Journal of Fisheries and Aquatic Science, 59:1819-1833.

Rohlf, F.J. 2005. tpsRelw, relative warps analysis, version 1.42. Department of Ecology and Evolution, State University of New York at Stony Brook.

Rohlf, F.J. 2005. tpsRegr, shape regression, version 1.31. Department of Ecology and Evolution, State University of New York at Stony Brook.

Rohlf, F.J. 2004. tpsDig, digitize landmarks and outlines, version 2.0. Department of Ecology and Evolution, State University of New York at Stony Brook.

Sage, R.D. and Selander R.K. 1975. Trophic radiation through polymorphism in cichlid fishes. Proceedings of the National Academy of Sciences, 72:4669-4673.

Sanderson, S.L., Cech, J.J., and Patterson, M.R. 1991. Fluid dynamics in suspension-feeding blackfish. Science, 251:1346-1348.

Sandlund, O.T., Gunnarsson K., Jonasson P.M., Jonsson B., Lindem T., Magnusson K.P., Malmquist H.J., Sigurjonsdottir H., Skulason S., and Snorrason S. 1992. The arctic charr Salvelinus alpinus in Thingvallavatn. Oikos, 64:305-351.

Schluter, D. 1995. Adaptive radiation in sticklebacks – trade-offs in feeding performance and growth. Ecology, 76:82-90.

Schluter, D. 1996. Ecological speciation in postglacial fishes. Philosophical Transactions of the Royal Society of London, 351:807-814.

Schluter, D. 2000. The ecology of adaptive radiation. Oxford University Press, Oxford. 288pp.

Schluter, D. 2001. Ecology and the origin of species. Trends in Ecology and Evolution, 16:372-380.

Schluter, D. and McPhail J.D. 1992. Ecological character displacement and speciation in sticklebacks. American Naturalist, 140:85-108.

Skulason, S. and Smith T.B. 1995. Resource polymorphisms in vertebrates. Trends in Ecology and Evolution, 10:366-370.

Smith, T.B. 1987. Bill size polymorphism and intraspecific niche utilization in an African finch. Nature, 329:717-719.

Snorrason, S.S., Skulason, S., Jonsson, B., Malmquist, H.J., Jonasson, P.M., Sandlund, O.T., and Lindem, T. 1994. Trophic specialization in Arctic charr *Salvelinus alpinus* (Pisces; Salmonidae): morphological divergence and ontogenetic niche shifts. Biological Journal of the Linnean Society, 52:1-18.

Speciation. 2001. Trends in Ecology and Evolution; special issue. Vol. 16, no. 7.

Stockwell, C.A. and Weeks, S.C. 1999. Translocations and rapid evolutionary responses in recently established populations of western mosquitofish (*Gambusia affinis*). Animal Conservation, 2:103-110.

Stockwell, C.A. and Leberg, P.L. 2002. Ecological genetic and the translocation of native fishes: emerging experimental approaches. Western North American Naturalist, 62:32-38.

Stockwell, C.A., Hendry, A.P. and Kinnison, M.T. 2003. Contemporary evolution meets conservation biology. Trends in Ecology & Evolution, 18:94-101.

Streelman, J.T., Gmyrek, S.L., Kidd, M.R., Kidd, C., Robinson, L, Hert, E., Ambali, A.J. and Kocher, T.D. 2004. Hybridization and contemporary evolution in an introduced cichlid fish from Lake Malawi National Park. Molecular Ecology, 13:2471-2479.

Taylor, E.B. and Bentzen P. 1993. Evidence for multiple origins and sympatric divergence of trophic ecotypes of smelt (*Osmerus*) in northeastern North America. Evolution, 47:813-832.

Trussell, G.C. and Etter, R.J. 2001. Integrating genetic and environmental forces that shape the evolution of geographic variation in a marine snail. Genetica, 112:321-337.

Walker, J.A. 1997. Ecological morphology of lacustrine threespine stickleback *Gasterosteus aculeatus* L (Gasterosteidae) body shape. Biological Journal of the Linnean Society, 61:3-50.

Waters, C.A. 1960. A study of the life history and taxonomic position of the blueback trout, *Salvelinus oquassa* (Girard). M.Sc. Thesis, University of Maine, Orono. 90pp.

Webb, P.W. 1975. Hydrodynamics and energetics of fish propulsion. Bulletin of the Fisheries Research Board of Canada, 190:1-159.

Webb, P.W. 1984. Body form, locomotion and foraging in aquatic vertebrates. American Zoologist 24:107-120.

Wootton, R.J. 1998. Ecology of Teleost Fishes. London: Kluwer Press.

APPENDIX – INDIVIDUAL POPULATION VON BERTALANFFY GROWTH FUNCTIONS AND SIZE-AT-AGE DATA

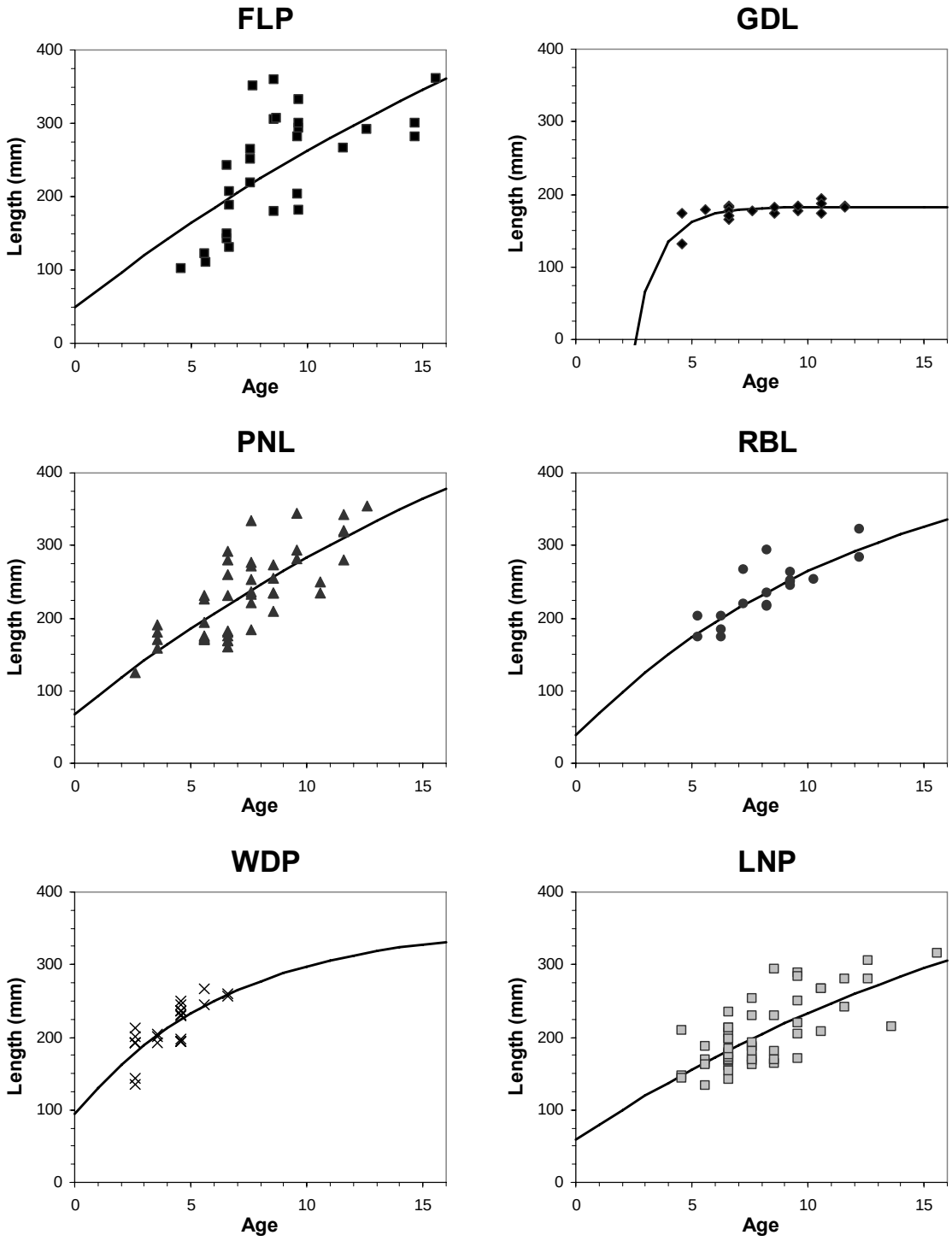

Figure A.1. Individual population VBGF curves with size-at-age data for each Arctic charr population included in this thesis.

BIOGRAPHY OF THE AUTHOR

Wendy Kay Michaud was born in Caribou, Maine on November 29, 1979. While growing up, she lived in Van Buren, East Millinocket, and Rumford, Maine, where she attended Mountain Valley High School. After high school, Wendy immediately entered college and received a Bachelor's degree in Secondary Education with a concentration in Biology from the University of Maine at Farmington in 2002. She then moved right into a Master's degree program in zoology at the University of Maine in the fall of that year. Wendy is a candidate for the Master of Science degree in Zoology from the University of Maine in August 2006.