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The Influence of Anadromous Alewife on Maine Lakes and Streams: Using Nutrient Limitation Assays and Stable Isotopes to Track Marine-Derived Nutrients

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THE INFLUENCE OF ANADROMOUS ALEWIFE ON MAINE LAKES AND STREAMS: USING NUTRIENT LIMITATION ASSAYS AND STABLE ISOTOPES TO TRACK MARINE-DERIVED NUTRIENTS

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By Katie Norris

Thesis Advisor Dr. Kevin Simon

An Abstract of the Thesis Presented
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Anadromous fish can act as nutrient subsidies to freshwater ecosystems when they return there to spawn. However, relatively few studies have quantified the role of alewife (*Alosa pseudoharengus*) as an ecologically important source of marine-derived nutrients (MDN) to lakes and streams. Primary producers in lakes and streams are often limited by nutrients, such as nitrogen and phosphorus. If alewife bring nutrients to lakes and streams, then the limitation of primary producers in those systems should be alleviated. Nutrient limitation assays and stable isotopes were used to examine the effects of alewife MDN on Maine lakes and their outlet streams. Nutrient limitation assays were run prior to, during, and after alewife runs and again after lake turnover. Alewife runs increased water nutrient concentrations in streams slightly, but not in lakes. There was also no coherent shift or alleviation of nutrient limitation in alewife lakes and streams compared to non-alewife systems. There was enrichment in $\delta^{13}C$ and $\delta^{15}N$ of lake zooplankton and resident fish in one alewife lake in comparison to a non-alewife lake.

Additionally, white perch in Fields Pond, to which alewife access was restored by dam removal, were relatively more enriched in ^{15}N after alewife re-introduction in 2011. There was less of an alewife effect on freshwater nutrient limitation than was expected, but the current densities of alewife runs were relatively low compared to historical counts and other areas of the northeastern U.S. Results from the stable isotope data suggest that some MDN were incorporated into lake food webs. This study has relevance in Maine given the current and proposed dam removals and diadromous fish restoration, which will restore access to historical freshwater habitat for native anadromous alewife.

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Chapter 1

BACKGROUND

Anadromous fish spend their adult lives in the ocean and return to spawn in coastal freshwater streams and lakes. Because anadromous fish travel up rivers to spawn, they link the marine environment to upstream freshwater ecosystems. In the course of this migration, the fish transport nutrients from the ocean to inland streams and lakes. These nutrients are commonly referred to as marine-derived nutrients (MDN). Thus, anadromous fish can act as a nutrient resource subsidy to freshwater ecosystems (Flecker et al. 2010). In order to better understand the importance of anadromous fish to freshwater nutrient budgets researchers have studied both Pacific salmon (*Oncorhynchus* spp) in the western United States (e.g. Mitchell and Lamberti 2005, Chaloner et al. 2007, Kohler et al. 2008, and Ruegg et al. 2011) and alewife (*Alosa pseudoharengus*) in the Atlantic (e.g. Durbin et al. 1979, Garman and Macko 1998 and Post and Walters 2009).

The importance and influence of Pacific salmon on freshwater systems has been studied for much longer and in more depth than most other anadromous fishes. Pacific salmon accumulate about 95% of their biomass in the ocean, but migrate to freshwater to spawn. They are semelparous and die after spawning in freshwater streams, or sometimes lakes. This means they can be a large source of MDN to freshwater systems. The ecological significance of these MDN subsidies to lakes rearing sockeye salmon has been studied since at least the 1930s (Juday et al. 1932, Naiman et al 2002). More recent work has revealed a similar subsidy effect in streams and terrestrial riparian zones (compiled works in Naiman et al. 2002 and Willson et al. 2004). Such studies often quantify changes in nutrient concentrations and ecological parameters (e.g. algal standing stocks)

or use stable isotopes to detect the incorporation of MDN into freshwater food webs (Willson et al. 2004, Janetski et al. 2009). While studying in-stream effects, Mitchell and Lamberti (2005) found that salmon increased stream water nitrogen (as NH_4^+) on average 3-fold and phosphorus (as soluble reactive phosphorus, SRP) by 10-fold compared to levels before spawning runs. Additionally, salmon subsidies can stimulate primary productivity in streams (Chaloner er al. 2007) and alleviate nutrient limitation (Ruegg et al. 2011), but this is often tempered by environmental factors such as run density, light, water temperature and discharge. It is clear then that Pacific salmon can be a resource subsidy to freshwater systems, however, the magnitude of salmon influence varies across studies and systems (Janetski et al. 2009) and can be complicated by their other role as an ecosystem engineer in building stream-bed spawning redds (Ruegg et al. 2012).

In comparison to Pacific salmon, alewife in the northeast U.S. have not been studied as thoroughly or for as long. Perhaps this disparity is partially due to the iteroparous life history of alewife as opposed to the semelparity of salmon that leads to a dramatic mass senescence, and the larger size of salmon runs compared to small current runs of alewife. Regardless, alewife do contribute MDN from the ocean to freshwater systems with some evidence of ecological consequence (Durbin et al. 1979, Garman and Macko 1998, MacAvoy et al. 2009, Walters et al. 2009). These nutrients brought by alewife were found to stimulate leaf litter respiration (Durbin et al. 1979) and subsidize food webs to a small degree (Walters et al. 2009). MDN from alewife were also found in piscivorous fish via stable isotope analysis (Garman and Macko 1998, MacAvoy et al. 2009). Such studies on both alewife and Pacific salmon provide valuable information

about research methods and the current understanding of anadromous fish impacts on freshwater ecosystems.

Alewife range from Newfoundland to North Carolina (Greene et al. 2009) and runs that were once extremely numerous have declined by 99.9% in large northeastern U.S. rivers (Limburg and Waldman 2009). This decline in alewife populations is mostly due to the construction of dams that block passage to streams and lakes (Hall et al. 2010). Recently, the National Marine Fisheries Service has listed alewife as a 'species of concern' (NOAA 2009). Ecologically alewife have co-evolved with other diadromous fish, and in large numbers may provide cover for outmigrating Atlantic salmon smolts (Saunders et al. 2006). Economically, alewife have been an important baitfish for Maine lobster fisheries (MDMR 2008). Through restoration efforts, including the implementation of fish ladders and dam removal, alewife are regaining access to parts of their historical spawning habitats. This return of previously blocked alewife to freshwater systems may have important ecological impacts in lake and stream ecosystems. Currently, the magnitude of the effect of alewife to freshwater systems is not well understood. Quantifying alewife effects will inform resource managers and agencies to better manage these systems. Because of their dynamic lifecycle and historical importance, the restoration of alewife populations to freshwater systems in Maine is important both ecologically and economically (Saunders et al. 2006). There are few published studies (Havey 1961, Kircheis et al. 2002, Saunders et al. 2006) that examine Maine's alewife populations and their impact on Maine freshwater ecosystems.

There has also been growing interest in trying to understand ecosystem linkages, including marine to freshwater exchanges and the role that diadromous fish play as a

connecting link (Lamberti et al. 2010). While the marine-freshwater link has been well explored in some cases, less attention has been paid to linkages among freshwater systems. Thus, the goal of this study was to measure the ecological effects of alewife nutrient subsidies to lakes and the outlet streams that drain them. I took two approaches to address the above objective and these are described in the following chapters. In Chapter 2, I used nutrient limitation assays in lakes and their outlet streams to quantify the effect of alewife as an ecosystem nutrient subsidy. Additionally, I examined the coherency of limitation patterns in linked lake-stream systems. Next, in Chapter 3, I measured the abundance of stable isotopes of carbon, nitrogen and sulfur (^{13}C, ^{15}N, and ^{34}S) in lake organisms to detect marine-derived nutrients brought by alewife. This method tracked the incorporation of these nutrients into higher lake trophic levels. Finally, I synthesized information from both approaches in Chapter 4 and discuss the larger picture of alewife effects on freshwater ecosystems.

Chapter 2

NUTRIENT LIMITATION PATTERNS AND LAKE-STREAM LINKAGES

2.1 Introduction

Anadromous fish are key vectors in linking marine, riverine, and lake ecosystems (Greene et al. 2009). With the construction of dams on many rivers and lake outlets, the longitudinal connectivity vital to anadromous fish migration has been altered or eliminated altogether (Hall et al. 2010). In the case of alewife (*Alosa pseudoharengus*), this has caused declines in both available habitat and overall alewife populations in Maine since 1634 (Hall et al. 2010). Only since about the 1980s has there been scientific research specifically investigating linkages between aquatic ecosystems (Vannote et al. 1980, Lamberti et al. 2010). While studies of ecosystem linkages are not yet abundant, some research is moving beyond an ecosystem-specific focus and recognizing the need for further study in the area of ecosystem linkages (Lamberti et al. 2010).

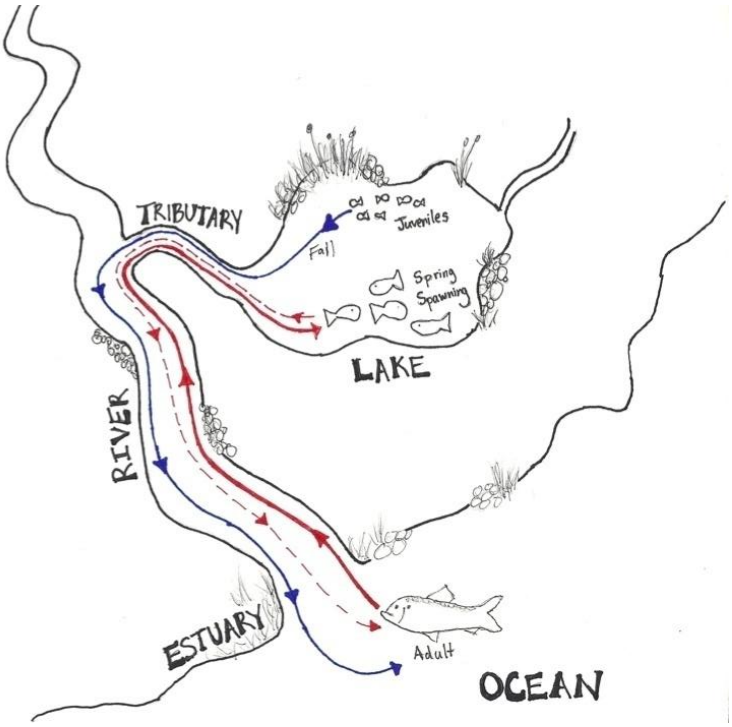
Alewife act as a biological linkage when they physically move from one system to another – in this case from oceans, through rivers to lakes. In a synthesis of migratory fish effects Flecker et al. (2010) discussed how the concept of food web subsidies is a useful way to assess the ecological importance of migratory fish. They noted two categories of effects that migratory fish can have on food webs of the receiving system, including 1) material subsidies (e.g. MDN), and 2) changes to ecosystem processes (e.g. predation of zooplankton or competition). Alewife are likely to act via both pathways as they can deliver MDN to freshwater systems (Durbin et al. 1979, MacAvoy 2009) and have strong trophic effects through predation of zooplankton in lakes (Brooks and

Dodson 1965, Post et al. 2008). Flecker et al. (2010) also describe the factors that affect the magnitude of a subsidy brought by migratory fish. These factors include the biomass of fish, prior availability of nutrients in the receiving ecosystem, and a mechanism for liberating the subsidy brought by the fish. Migratory fish are more than just agents for transporting nutrients; they can also directly alter ecosystem processes. For example, Pacific salmon alter streambeds and sediment transport when digging spawning redds (Flecker et al. 2010). By migrating between aquatic systems fish, including alewife, link different ecosystems and have the potential to subsidize and alter the receiving ecosystem.

Every spring adult alewife migrate from the ocean to coastal freshwater rivers of the northeastern U.S. These fish swim upstream to spawn in lakes and ponds (Figure 1), often returning to their natal lake. When alewife return to spawning lakes via streams they may increase the nutrient (e.g. nitrogen and phosphorus) and energy (e.g. carbon) availability in these freshwater systems. This occurs primarily through three major avenues: excretion, release of gametes, and post-spawning mortality of the fish. Alewife are iteroparous though, meaning some adults die but others return to the ocean after spawning. This life history mode differs from that of semelparous salmon, in which all the adults die after spawning. This difference in life history may limit the magnitude of the alewife MDN subsidy to freshwaters. Young-of-the-year (YOY) alewife spend the summer in their natal pond or lake growing and eating zooplankton (Brooks and Dodson 1965, Post et al. 2008). A seasonal decrease in zooplankton body size due to juvenile predation can be used as an indicator of anadromous alewife presence. After one summer the YOY juvenile alewife migrate out of the lake and downstream to the ocean, where

they continue to grow for 3-5 years into adults (Figure 1). If they escape predation in the ocean, these fish will then return in the spring to their natal lake, thus completing the alewife life cycle.

Figure 1. Diagram of anadromous alewife life cycle. Adults migrate upstream in spring (solid red line), spawn in lakes and some return shortly thereafter to the ocean (dashed red line). Juvenile young-of-the-year (YOY) grow in the lake until late summer and then migrate downstream out to the ocean (solid blue line).



Several studies have attempted to quantify the amount and importance of alewife nutrient contributions to freshwater lakes and streams. Durbin et al. (1979) combined mean carbon (C), nitrogen (N) and phosphorus (P) content of adult alewife carcasses and excretion with adult run size to estimate nutrient delivery to one pond. They estimated that alewife brought 453×10^4 g C, 72.8×10^4g N, and 11.5×10^4 g P to Pausacaco Pond, Rhode Island during April and May of 1974 (Durbin et al. 1979). Carcasses, from post-

spawning mortality, accounted for most of the C, while excretion was a large source of the N brought by the alewife (Durbin et al. 1979). They estimated that biomass brought through alewife mortality (expressed per unit area or volume of lake water) could exceed Pacific salmon runs by 1-2 orders of magnitude (Durbin et al. 1979). The ecological consequence of alewife nutrients was an increase in the rate of leaf litter decomposition in streams due to simulated microbial activity, presumably a result of elevated nutrient availability.

Excretion from spawning alewife as they travel up streams and in lakes is potentially important because alewife excrete nutrients as ammonium, NH_4^+, which is immediately available for uptake by other aquatic organisms (Vanni 2002). Post and Walters (2009) measured short- and long-term mass-specific nutrient excretion rates for spawning alewife in Bride Lake, Connecticut. Alewife excreted an average of 24.71 μg N per gram wet fish mass per hour (~75% as NH_4^+), and 2.17 μg P per gram wet fish mass per hour. This provided the first calculated excretion rates of N and P per alewife. In another study at Bride Lake, Connecticut, West et al. (2010) investigated the net nutrient loading of alewife across a range of theoretical population sizes. They quantified the P budget at Bride Lake including loading from incoming adults and export of P by young-of-the-year. These fluxes were compared to an estimated watershed background nutrient-loading of the lake. Alewife contributed 23% of the total P input to Bride Lake during the study, and based on counts from historic alewife runs the fish could have imported around 44% in the 1960s when returning alewife were 80% more abundant (West et al. 2010). From their models and experiments West et al. (2009) concluded that alewife are rarely net nutrient exporters for lakes, except if the returning adult population is small

and the YOY population is large. Direct measurements of the effects of these P inputs were not measured by West et al. (2010) but they proposed that unless managed closely, alewife could exacerbate lake eutrophication in already nutrient-rich systems.

While research on alewife has provided valuable information about their potential role as nutrient sources, the methods used have limitations, particularly in regards to the ecological consequences of such nutrient input. Calculating a nutrient budget for a system is not only a lengthy process with the potential for missing components, it is also site specific and not usually applicable broadly. Implicit in this method is the assumption that anadromous fish bring a needed subsidy to nutrient limited systems without testing this assumption directly. An alternative method to understanding the influence of anadromous fish is to determine the initial nutrient limitation of the primary producers in a freshwater system and then measure if N and P brought by returning fish relax or change the limitation (Ruegg et al. 2011).

Ecosystem subsidies, such as those brought by anadromous fish, are only ecologically valuable to the receiving system if there exists a need, often measured as the nutrient limitation, for the resources the subsidy supplies (Marczak et al. 2007, Flecker et al. 2010). Biological productivity of an ecosystem is often determined by the availability of key nutrients, in particular N and P (Schindler 1977, Tilman et al. 1982, Allan and Castillo 2007, Elser et al. 2007). The high demand for N and P relative to their availabilities makes them a limiting resource, which can limit biological activity (Tilman et al. 1982, Allan and Castillo 2007). Autotrophs in particular are often limited by N and/or P, including benthic algae in streams (Allan and Castillo 2007) and phytoplankton in lakes (Tilman et al. 1982). Nutrient limitation of a system is often measured

empirically by testing the response of autotrophs to addition of a known quantity of N and/or P. Many studies have determined the identity of limiting nutrients and the magnitude of limitation in a stream or lake (e.g. Doyle et al. 2005, Chaloner et al. 2007, Marcarelli and Wurtsbaugh 2007, Sanderson et al. 2009). However, measuring changes to nutrient limitation of a system has rarely been done in the context of MDN from migratory fish (but see Ruegg et al. 2011).

In a meta-analysis Marczak et al. (2007) found that most ecosystem resource subsidy studies did not explicitly consider if the organisms in the receiving ecosystem needed the subsidy. With this in mind, Ruegg et al. (2011) found that autotrophic biofilm nutrient limitation in nutrient-poor streams in southeast Alaska was alleviated after salmon returned. To accurately evaluate importance of alewife nutrient subsidies to lakes and streams, the pre-subsidy nutrient limitation should be examined in addition to measuring the amounts of N and P imported. Such a study has not been done for alewife to examine the relative importance of their subsidy contribution to freshwater lakes and streams.

Anadromous fish may be potential nutrient sources to both lakes (Durbin et al. 1979, West et al. 2010) and streams (Walters et al. 2009, Ruegg et al. 2011), yet rarely are both systems considered in tandem. Such an approach may be particularly useful considering important linkages occur between lakes and their respective outlet streams, which include both physical and chemical linkages (Lamberti et al. 2010). Some studies have examined such linkages in chains of lake-stream systems (Wurtsbaugh et al. 2005, Marcarelli and Wurtsbaugh 2007). Wurtsbaugh et al. (2005) measured nutrient fluxes into and out of linked lakes and streams and found that lakes have the potential to

mitigate nutrient pulses to downstream biota, making the nutrients available over a longer period of time. In the context of fish delivery of MDN, lakes may therefore modulate the magnitude and timing of nutrient subsidy to streams. In a similar study, Marcarelli and Wurtsbaugh (2007) used bioassays to measure stream nutrient limitation in relation to position either upstream or downstream of a lake. They found that lakes and their associated outlet streams differed in their respective nutrient limitation patterns. If lakes and outlet streams are inherently limited by different nutrients, then the importance or identity of the nutrient subsidy (e.g. C, N, or P) provided by alewife may differ in lakes or streams. However, Wurtsbaugh et al. (2005) noted the lack of studies that view lakes and streams as integrated systems. More research is needed on lake-stream linkages in general and across lakes of differing trophic states and geographic regions to better understand lake-stream nutrient dynamics.

I measured the ecological effect of alewife nutrient subsidies, using nutrient limitation assays, in Maine lakes and streams. To accomplish this, nutrient limitation assays were run in lakes and associated outlet streams before, during, and after alewife runs. This nutrient limitation assay approach is valuable in two important ways: it identifies if the primary producers in the freshwater system are nutrient limited initially and by which nutrient(s), and it provides a straightforward way of tracking the magnitude of the influence of alewife nutrient subsidies

I hypothesized that if anadromous alewife subsidize freshwater streams and lakes when they return in the spring to spawn, then the nature and magnitude of nutrient limitation of primary producers will be shifted or alleviated. In terms of lake and stream

connectivity I predicted that upstream lakes would influence the initial nutrient inputs to outlet streams but would not dictate limitation patterns.

2.2 Methods

I sampled 7 lakes and their respective outlet streams where alewife were present, and 7 lakes and outlet streams without alewife runs in central and coastal Maine, USA (Figure 2). To gauge the potential of alewife to deliver ecologically meaningful MDN subsidies, I performed a series of nutrient limitation assays in the lakes and outlet streams. Nutrient limitation assays for both the lakes and streams were performed prior to (April in streams, May in lakes), during (May in streams, June in lakes) and after (August for both) alewife runs and again after lake turnover (October for both). The timing of alewife runs in these systems was determined using historical records and collaboration with agencies and individuals that performed counts of the returning alewife. The approach I used allowed for comparisons of nutrient limitation for pre/post alewife presence, seasonal variations, and lake-stream synchronicity.

2.2.1 Study Sites

Lakes ranged in size from 42 hectares (ha) to 1,191 ha (Table 1). Sites ranged from central Maine, near Etna, southeast to Mount Desert Island on the central coast of Maine (Figure 2). All field sites are located within the Laurentian Plains and Hills level III ecoregion of Maine (EPA 2010). If possible, systems with alewife counts were used. All non-alewife streams had barriers downstream of the sampling location that blocked migratory fish movement at the time of the study. Alewife counts for Fields Pond were

collected by National Oceanic and Atmospheric Association staff, for Somes and Long Ponds by visual counts conducted by volunteers of the Somes-Meynell Wildlife Sanctuary. Acadia National Park staff conducted visual counts of alewife entering Seal Cove Pond. Estimates of the alewife density in Alamoosook Lake and Toddy Pond came from the Maine Atlantic States Marine Fisheries Commission Report for 2010, which has numbers for both alewife harvested and the required number of fish that must be allowed to pass into the lakes (Maine ASMFC 2010).

Figure 2. Map of field sites in Maine, USA. Sites with alewife runs are labeled with red markers and non-alewife sites are blue markers. Background map from Googlemaps.

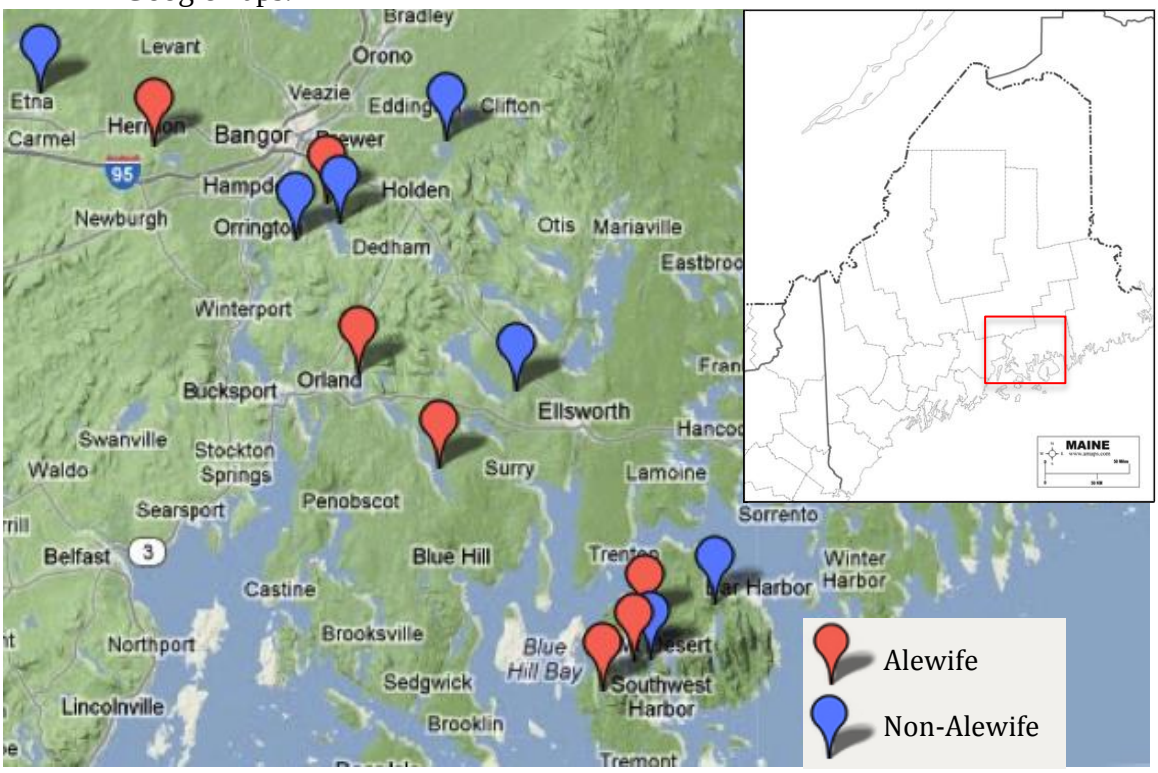


Table 1. Location and physical characteristics of lake field sites. Areas given in hectares (ha), depths in meters (m), volumes in cubic meters (m^3), and fish density in number of fish per volume of water. N/A means no data. Areas, depths and volumes provided by Maine DEP. Alewife count density calculated from 2011 data provided by NOAA, Acadia National Park, Somes-Meynell Wildlife Refuge, and the Maine Atlantic States Marine Fishery Commission Sustainable Fishing Plan.

Lake Name	Latitude/Longitude	Area (ha)	Mean Depth (m)	Maximum Depth (m)	Volume (m^3)	Alewife Density (#fish/m^3)
Alewife						
Hermon	44.780 -68.935	187	3.0	5.2	$5.1x10^6$	No Data
Fields	44.731 -68.746	210	4.0	9.4	$2.3x10^6$	0.00007
Somes	44.360 -68.347	42	3.3	7.6	$1.1x10^6$	0.01137
Alamoosook	44.579 -68.700	403	4.9	8.5	$1.8x10^7$	0.00194
Seal Cove	44.302 -68.397	115	5.5	13.4	$3.9x10^6$	0.00014
Long	44.327 -68.361	363	11.2	34.4	$3.3x10^7$	0.00003
Toddy	44.528 -68.620	974	8.2	37.2	$6.1x10^7$	0.00008
Non-Alewife						
Etna	44.828 -69.100	146	1.8	3.7	$2.3x10^6$	N/A
Davis	44.786 -68.593	204	3.0	4.3	$3.6x10^6$	N/A
Swetts	44.699 -68.780	50	3.4	10.4	$1.0x10^6$	N/A
Brewer	44.710 -68.726	388	7.9	14.6	$2.8x10^7$	N/A
Echo	44.328 -68.337	96	8.5	20.1	$6.2x10^6$	N/A
Eagle	44.362 -68.250	189	13.4	33.5	$2.2x10^7$	N/A
Branch	44.594 -68.559	1191	11.9	37.8	$1.0x10^8$	N/A

2.2.2 Sample Collection and Analysis

On each of the sampling dates chemical and physical variables were measured near the maximum depth of the lake and in the outlet stream between 30m and 300m from the outlet of the lake (with one exception of 700m in the outlet of Echo). GPS coordinates were recorded for each site location with a handheld GPS unit (Garmin model GPS76). At these sampling locations both lake (at ~2m depth) and stream water samples were collected in 60mL acid-washed bottles, filtered through Whatman GF/F filters, kept on ice for transport and refrigerated (1-4 weeks) until analysis. These samples were analyzed for soluble reactive phosphorus (SRP) and nitrate (NO_3^-) concentrations

on a Lachat QuickChem® 8500 Flow Injection Analysis System (APHA 2005). The limit of quantification (LOQ) was 2µg/L for SRP, and 1µg/L for nitrate. Ammonium (NH_4^+) concentration of these same samples was measured using the fluorometric method (Taylor et al. 2007, LOQ 6µg NH_4-N/L). Separate unfiltered water samples were collected and frozen until analysis for total nitrogen (TN) and total phosphorus (TP) concentration using the persulfate digestion method (APHA 2005) for simultaneous determination of TN and TP on a Latchat QuickChem® 8500 Flow Injection Analysis System with a LOQ of 50µg/L for TN and 5 µg/L for TP. Lakes were sampled once per sampling period (May, June, August, and October). Sampling included a depth, temperature, and dissolved oxygen (DO) profile at 1-meter intervals with a YSI meter (YSI, Inc., model YSI85) (Appendix Figure A.3.). Secchi depth was measured using a 20 cm diameter black/white disk on the shaded side of the boat with an underwater view scope. Zooplankton were collected on each date by combining three tows using a Wildco® Wisconsin model plankton net with 80µm mesh to a depth 1-2m from the lake bottom. The three tows were combined and zooplankton were anesthetized with Alka-Seltzer®, preserved in 70% alcohol, and identified and counted in the laboratory. Between 175-200 individuals were identified, photographed, and measured using Image J software (http://rsb.info.nih.gov/ij/). Average body length for each genus of cladoceran was determined by measuring from the top of the head to the base of the tail spine. These zooplankton samples were collected because juvenile alewife are size-selective grazers (Brooks and Dodson 1965, Post 2008) on lake zooplankton and decreases in mean zooplankton body size can be used to confirm adult alewife entrance into lakes and trophic effect of juvenile alewife on zooplankton.

Streams were sampled when nutrient diffusing racks were deployed and collected. In streams, a handheld multi-meter (Hach®, model HQ40d) was used to measure pH, conductivity, dissolved oxygen, and temperature. After leaf-out, canopy cover was measured using a standard forestry spherical convex densitometer.

2.2.3 Nutrient Limitation Assays

Nutrient limitation of phytoplankton in lakes and benthic algae in streams was measured with nutrient addition assays, involving factorial addition of N and P. Limitation was measured in lakes using the microcosm 1-L container method (similar to Doyle et al. 2005 and Saros et al. 2005). Water from the epilimnion of each lake (~2m) was collected, filtered through a 150-μm mesh to remove zooplankton and transported in 18.9L plastic containers to the laboratory. In the laboratory, 1L of water was divided into 16, 1-L cubitainers, which were assigned randomly among four treatments. The four treatments for the lake samples were: no nutrients (control), 8μM N as NH_4NO_3 (+N), 0.5μM P as KH_2PO_4 (+P), or 8μM N as NH_4NO_3 + 0.5μM P as KH_2PO_4 (+N+P). There were 4 replicates of each treatment for each lake. All 1-L cubitainers from all study lakes were placed in a growth chamber (Percival-Scientific, Intellus environmental controller, model: 166LLVL) and incubated for 10 days at the average seasonal lake temperature (May: 11°C, June: 18°C, August: 24°C, October: 20°C) and a 14/10 (Day/Night) light cycle under 20W fluorescent tube lights (Figure 3). Aliquots from the cubitainers, between 200 and 700mL, were then filtered through a filter (Whatman GF/F). The filters were frozen (~21-56 days), thawed, ground, and then analyzed for chlorophyll *a* (chl*a*). Chl*a* was extracted in acetone overnight, analyzed by spectrophotometry on a Thermo

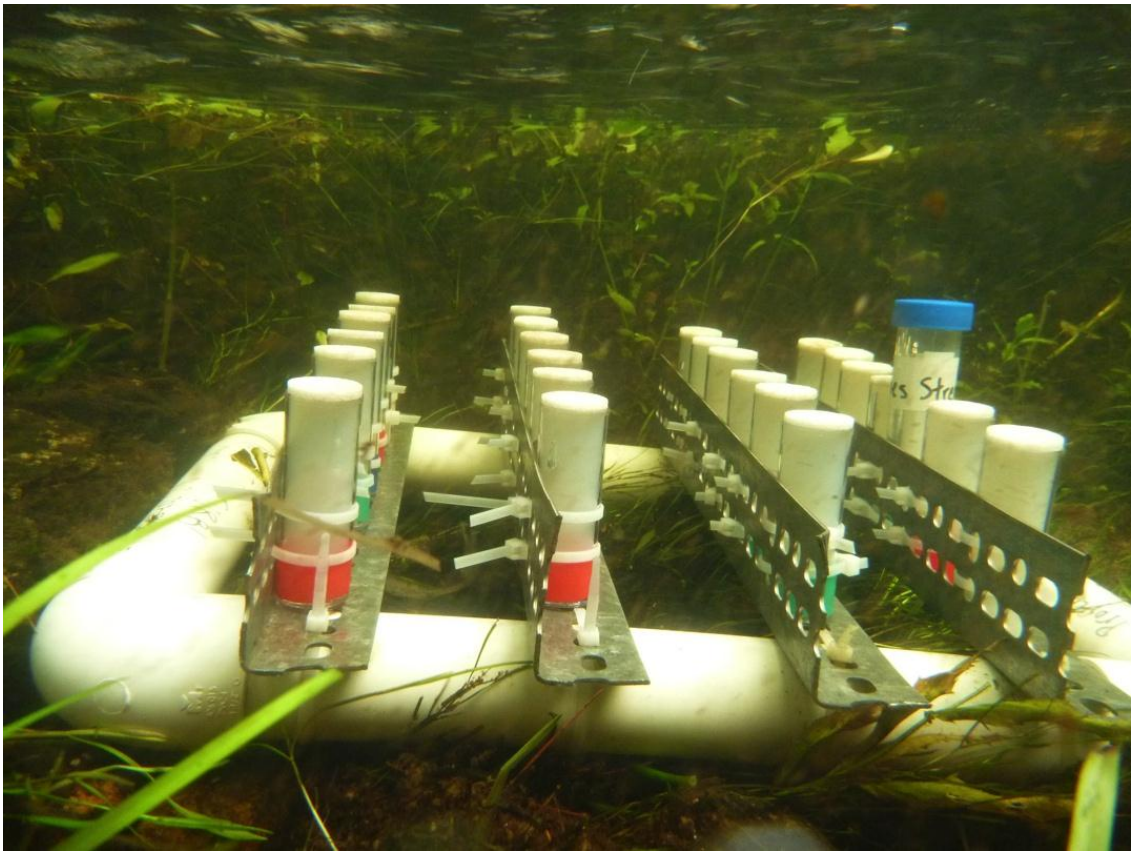
Scientific® Aquamate spectrophotometer and corrected for phaeopigments using an addition of HCl (APHA 2005).

Figure 3. Image of lake 1-L cubitainers in lab climate controlled growth chamber. See text for details.

Nutrient diffusing substrates (NDS) were used in streams to test if the growth of biofilms was nutrient-limited (Tank et al. 2006). NDS are a common method in stream ecology to measure algal response to nutrient additions (Francoeur et al. 1999, Marcarelli and Wurtsbaugh 2007, Rugenski et al. 2008, Sanderson et al. 2009). NDS contained bacterial-grade agar amended with one of four treatments: no nutrients (control), 0.5M N as NH_4NO_3 (+N), 0.2M P as KH_2PO_4 (+P), or 0.5M N as NH_4NO_3 + 0.2M P as KH_2PO_4 (N+P) (Tank et al. 2006, Sanderson et al. 2009). Porous silica fritted glass discs were fused to the diffuser cups (37mL polystyrene vials; Fisher Scientific 03-338-3D) to serve

as an inorganic substrate for algal growth (Tank et al. 2006, Sanderson et al. 2009). Each study stream had 6 replicates of each treatment per rack in haphazard arrangement (*N*=24 NDS per stream per rack) Racks were built from PVC and angle metal (Figure 4). I chose to place each rack in a run habitat under the best available canopy-gap, near the lake outlet (~50m-700m). These conditions were chosen to minimize interactions with groundwater upwelling/downwelling, and any effect of light differences among streams. These NDS assays were run for approximately 21 days *in situ*. After incubation, the NDS with algal growth were collected, placed in plastic bags by treatment, put on ice for transport, and frozen (~14-42 days) until analysis of chl*a* following the methods above. The discs were then placed in an aluminum dish, dried in a drying-oven overnight at 60°C, weighed, and then combusted to 500°C. After combustion discs were re-wetted, dried, and re-weighed to measure AFDM.. A total of 50 stream NDS assays were performed over four seasons. Two streams (Alamoosook and Swetts) did not have Pre data because the stream discharge declined during that experimental time period leaving the racks exposed. In addition, the outlet stream of Hermon Pond was not used in any sampling periods due to access issues.

Figure 4. Image of NDS rack *in situ*. Racks were anchored to the stream bottom with rebar stakes.



2.2.4 Data Analysis

Identity of the primary limiting nutrient of primary producers in each individual lake and stream for each season was determined using a two-way analysis of variance (ANOVA), with N and P as the main factors using the criteria from Tank et al. (2006). The limiting nutrient of heterotrophs, as determined by the AFDM, was also determined using this same analysis. The chl*a* and AFDM data were log-transformed prior to analysis to meet assumptions of normality and equal variance.

In order to gauge and compare the strength of nutrient limitation, I calculated a nutrient response ratio (RR) for N, P, and NP treatments (Ruegg et al. 2011). The RR of each stream or lake for each season was calculated using the following formula:

RR_x=[average chl*a* of treatment X / average chl*a* of control treatment]

A RR of 1 indicates no effect while values above and below 1 indicate stimulation or suppression by the nutrient. Thus, a RR indicates the magnitude of limitation in addition to which nutrient is primarily limiting productivity.

I used a two-way analysis of variance (ANOVA) with fish (alewife or non-alewife) and season (Pre/During/Post/Turnover) as factors to examine their effect on lake and stream dissolved inorganic nitrogen concentrations (DIN, calculated as the sum of the NO_3^- and NH_4^+concentrations), TP concentrations, RR-N, RR-P, RR-NP, response ratios of AFDM, and chl*a* of the controls as a proxy for biomass. If there was a significant interaction between fish and season, then this indicated that the effect of alewife depended on season, which was what I had predicted, or that the effect of season depended on fish. If a significant interaction was found then I split the data for the alewife and non-alewife systems. If there was no significant interaction then a Bonferroni post-hoc test on the original 2-way ANOVA was used to distinguish differences in RRs among seasons. I also examined seasonal patterns in the RRs of each lake or stream individually across all four seasons using separate 2-way ANOVAs (treatments and seasons as factors) with a Bonferroni post-hoc test. Because algal nutrient limitation is likely influenced by ambient nutrient availability I compared the magnitude of nutrient limitation (RR) with ambient concentrations of nutrients by regressing RR-N and RR-P with DIN and TP, respectively for lakes and streams separately. DIN vs. TP were

graphed for lakes, and streams, for each season to evaluate the theoretical nutrient limitation.

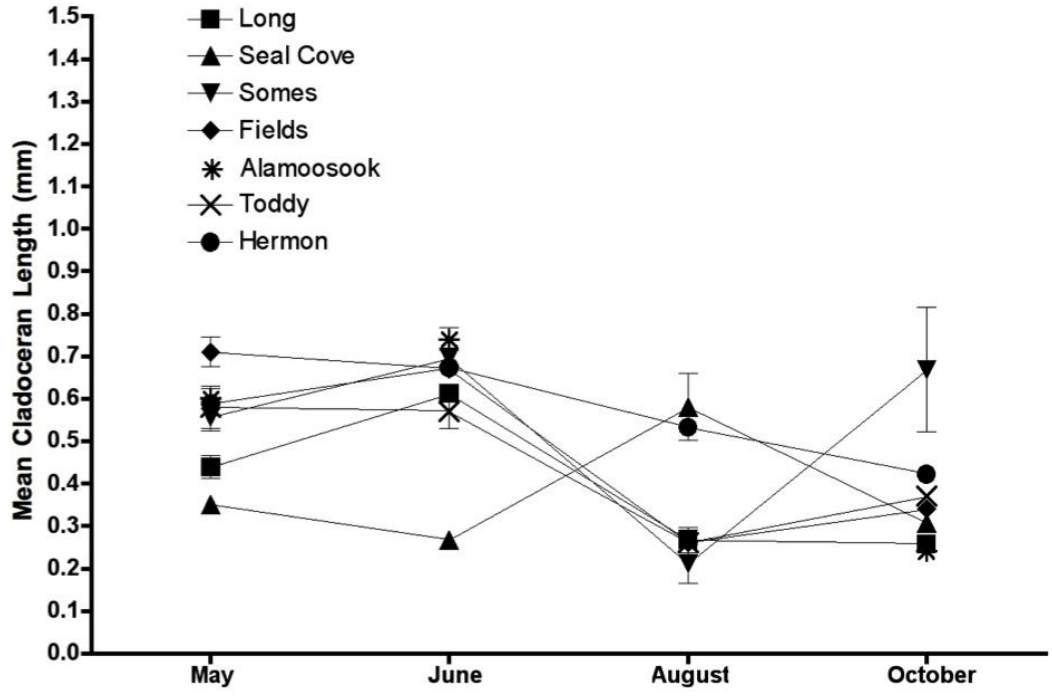
To examine lake-stream coherency, nutrient concentrations (DIN and TP) and RRs (N, P, and NP) of lakes and outlet streams were compared using Pearson's product-moment correlation. Statistical analyses were performed using GraphPad Prism, and R (for Mac OS X GUI 1.4) for the 2-way ANOVAs that determined the primary limiting nutrients. Results were considered statistically significant at $\alpha=0.05$ (or family-wise $\alpha=0.01$ for Bonferroni post-hoc comparisons).

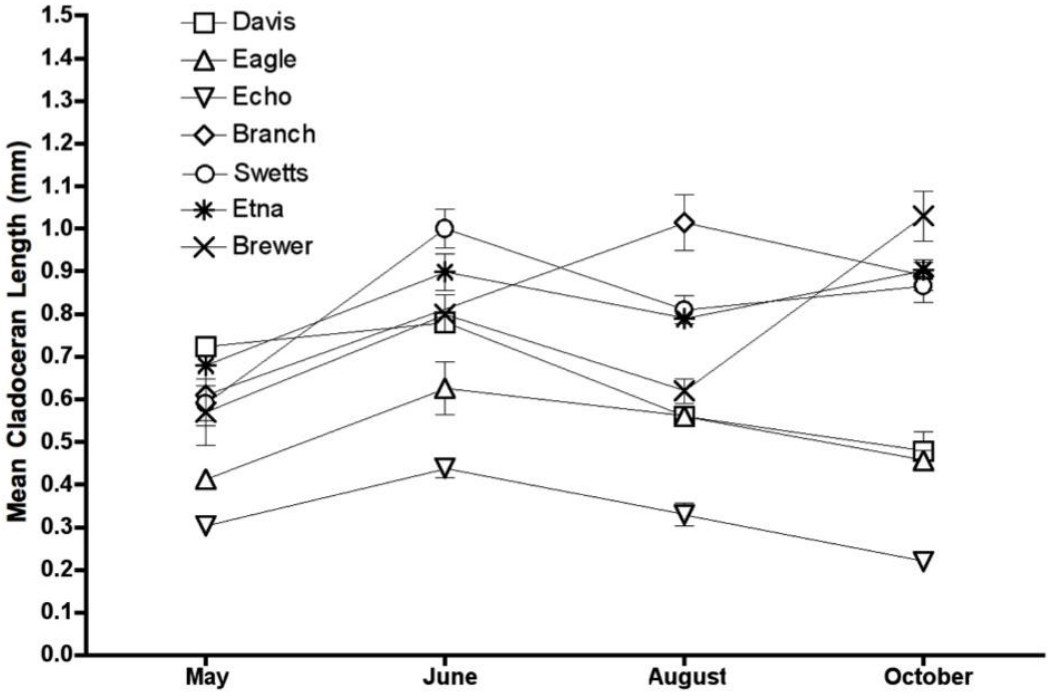
2.3 Results

2.3.1 Site Characteristics and Water Chemistry

Secchi disc depths for the lakes ranged from 2.5m to 6.7m in May, 2.4m to 9.9m in June, 2.6m to 10.5m in August, and 2.3m to 10.9m in October, with alewife lakes being shallower than non-alewife lakes overall (t-test, $P=0.034$) (Appendix A Table A.1.). Zooplankton body size was static or increased slightly over summer in lakes without alewife access (Figure 5). In most lakes with alewife access, zooplankton body size declined from June to August (Figure 5). Notable exceptions were Hermon and Seal Cove, where mean body size in Hermon only declined slightly from spring to fall, and it actually increased in Seal Cove (Figure 5).

Figure 5. Seasonal changes in mean cladoceran length. Mean cladoceran body lengths are given in mm ± standard error, for alewife lakes (A) and non-alewife lakes (B) from May through October.





The mean stream temperature, pH, dissolved oxygen (DO), conductivity, and percent canopy cover are shown in Table 2. Temperature ranged from an average of 13.3°C to 16.3°C, pH from 6.1 to 7.1, DO from 8.9mg/L to 10.3mg/L, conductivity from 27.8µS/cm to70.0µS/cm, and canopy cover from 12% open to 95%. There were no significant differences between alewife and non-alewife streams for these parameters (t-test; all $P>0.05$).

Table 2. Mean stream variables. Numbers are averages for all sampling periods except canopy cover, which was measured once after leaf out. (DO=dissolved oxygen)

Stream Name	Temperature (°C)	pH	DO (mg/L)	Conductivity (µS/cm)	Canopy cover (% open)
Alewife					
Fields	15.4	6.5	9.1	40.6	40
Somes	14.8	6.3	9.4	47.5	50
Alamoosook	16.3	7.0	10.2	46.0	95
Seal Cove	13.3	6.1	9.6	54.4	50
Long	14.4	6.5	9.8	41.7	23
Toddy	15.0	6.6	9.9	30.1	12
Mean	14.9	6.5	9.7	43.4	45
Non-Alewife					
Etna	14.5	6.8	9.0	70.0	50
Davis	16.1	7.0	9.5	57.8	19
Swetts	14.9	6.9	9.0	41.7	23
Brewer	14.6	7.1	10.0	29.3	76
Echo	14.6	6.4	8.9	50.5	18
Eagle	13.8	6.8	10.3	32.9	37
Branch	15.1	6.7	9.9	27.8	70
Mean	14.8	6.8	9.5	44.3	42

The DIN concentrations across all seasons were similar (t-test $P=0.56$) in alewife lakes (<1µg/L to 52µg/L), and non-alewife lakes (<1µg/L to 66µg/L) (Table 3). The DIN concentrations of streams were similar (t-test $P=0.84$) between alewife (<1µg/L to 78µg/L) and non-alewife (<1µg/L to 89µg/L with an outlier of 127µg/L) (Table 3). The

TP concentrations in alewife lakes were <2µg/L to 18µg/L and similar (t-test *P*=0.28) in non-alewife, <2µg/L to 21µg/L (Table 4). TP in streams was similar (t-test *P*=0.16) in alewife (<2µg/L to 22µg/L) and non-alewife (<2µg/L to 23µg/L) (Table 4). This is also illustrated in Figure 6.

Table 3. Lake and stream DIN concentrations for all sampling periods. All values are in µg/L and BDL means below the LOQ detection limit (<1µg/L). N/A means there were no data for that site/time.

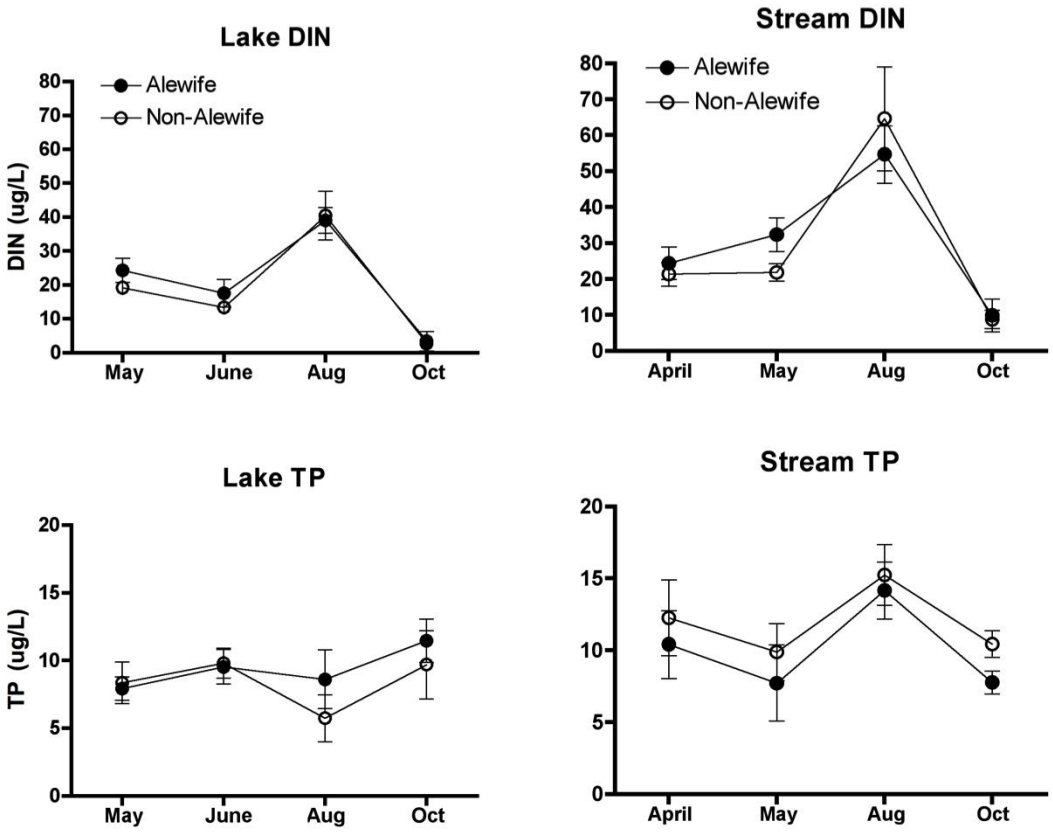
	Lakes				Streams			
Site	May	June	Aug.	Oct.	April	May	Aug.	Oct.
Alewife								
Somes	13	13	30	2	12	36	21	2
Long	33	30	48	BDL	34	44	59	5
SCP	20	11	49	BDL	15	13	78	BDL
Fields	14	9	29	2	19	28	68	29
Alamoosook	32	36	29	BDL	27	31	50	7
Toddy	36	16	52	BDL	39	43	53	18
Hermon	22	8	37	21	N/A	N/A	N/A	N/A
Mean	24	18	39	3	24	32	55	10
Non-Alewife								
Swetts	12	21	20	8	34	14	89	8
Brewer	17	12	66	2	32	23	29	14
Branch	26	13	17	BDL	17	13	56	BDL
Davis	17	14	28	BDL	11	31	87	9
Etna	23	12	43	7	20	23	48	21
Eagle	20	14	53	BDL	21	26	17	5
Echo	21	9	55	BDL	15	25	127	5
Mean	19	13	40	3	21	22	64	9

Table 4. Lake and stream TP concentrations for all sampling periods. All values are in μg/L and BDL means below the LOQ detection limit (<2μg/L). N/A means there were no data for that site/time.

	Lakes				Streams			
Site	May	June	Aug.	Oct.	April	May	Aug.	Oct.
Alewife								
Somes	8	8	8	11	8	9	10	9
Long	9	8	7	16	5	3	12	7
SCP	6	10	7	7	7	BDL	22	7
Fields	7	11	9	15	10	19	17	10
Alamoosook	10	9	BDL	9	21	9	14	9
Toddy	5	5	13	7	11	6	10	5
Hermon	12	16	18	16	N/A	N/A	N/A	N/A
Mean	9	10	10	10	10	8	14	8
Non-Alewife								
Swetts	16	12	11	21	19	18	12	11
Brewer	8	10	13	7	14	13	13	13
Branch	5	6	3	9	24	7	23	N/A
Davis	10	14	BDL	3	9	10	19	13
Etna	8	12	5	15	10	11	20	9
Eagle	4	7	4	10	5	BDL	11	7
Echo	6	8	5	2	7	10	8	9
Mean	7	9	3	8	12	10	15	11

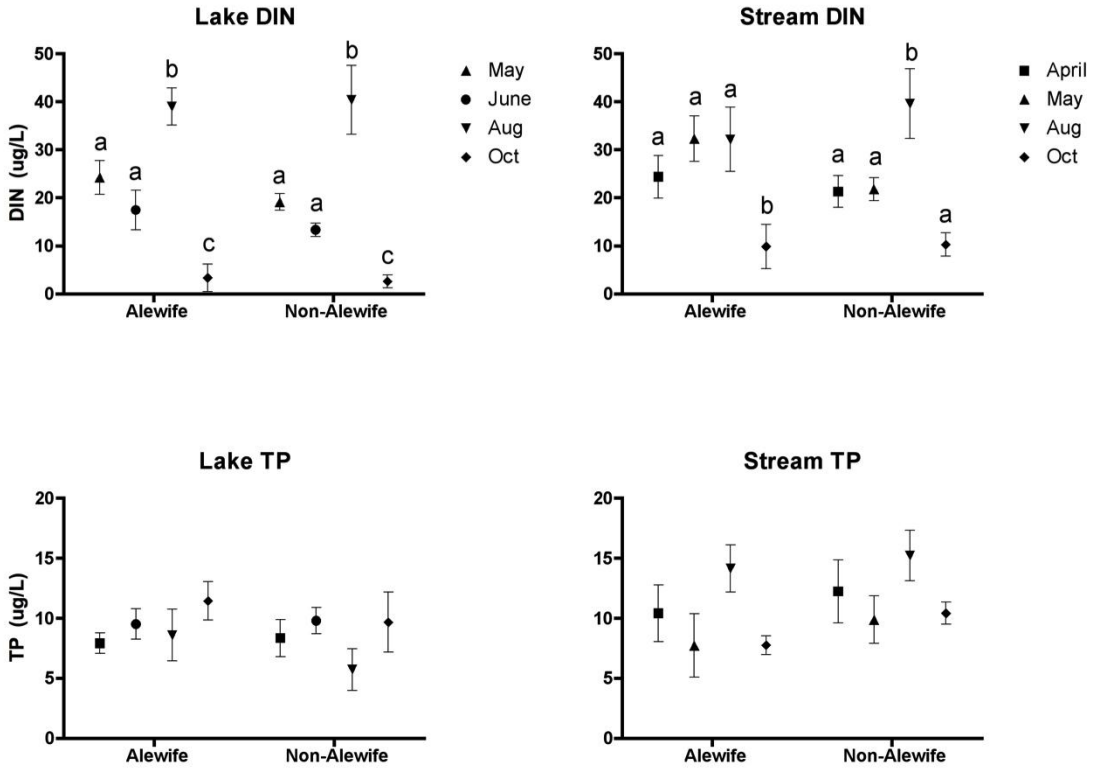
Lake and stream water DIN, and TP, generally followed the same temporal patterns in both alewife and non-alewife systems (Figure 6). DIN was mostly constant in April and May, increased in August, and then decreased in October. The exception to this pattern was in May during alewife presence where alewife streams had a marginally higher mean DIN concentration (P=0.06) of 32μg/L±4 compared to 22μg/L±2 in non-alewife streams. A similar increase was not seen with TP concentrations during runs. TP generally increased in lakes across the seasons, with stream TP also increasing until decreasing in October.

Figure 6. Lake and stream nutrient concentrations. The lake sampling periods correspond to Pre (May), During (June), Post (August) and Turnover (October) these are slightly behind the stream sampling periods as fish were in the streams before the lakes. Error bars are standard error.



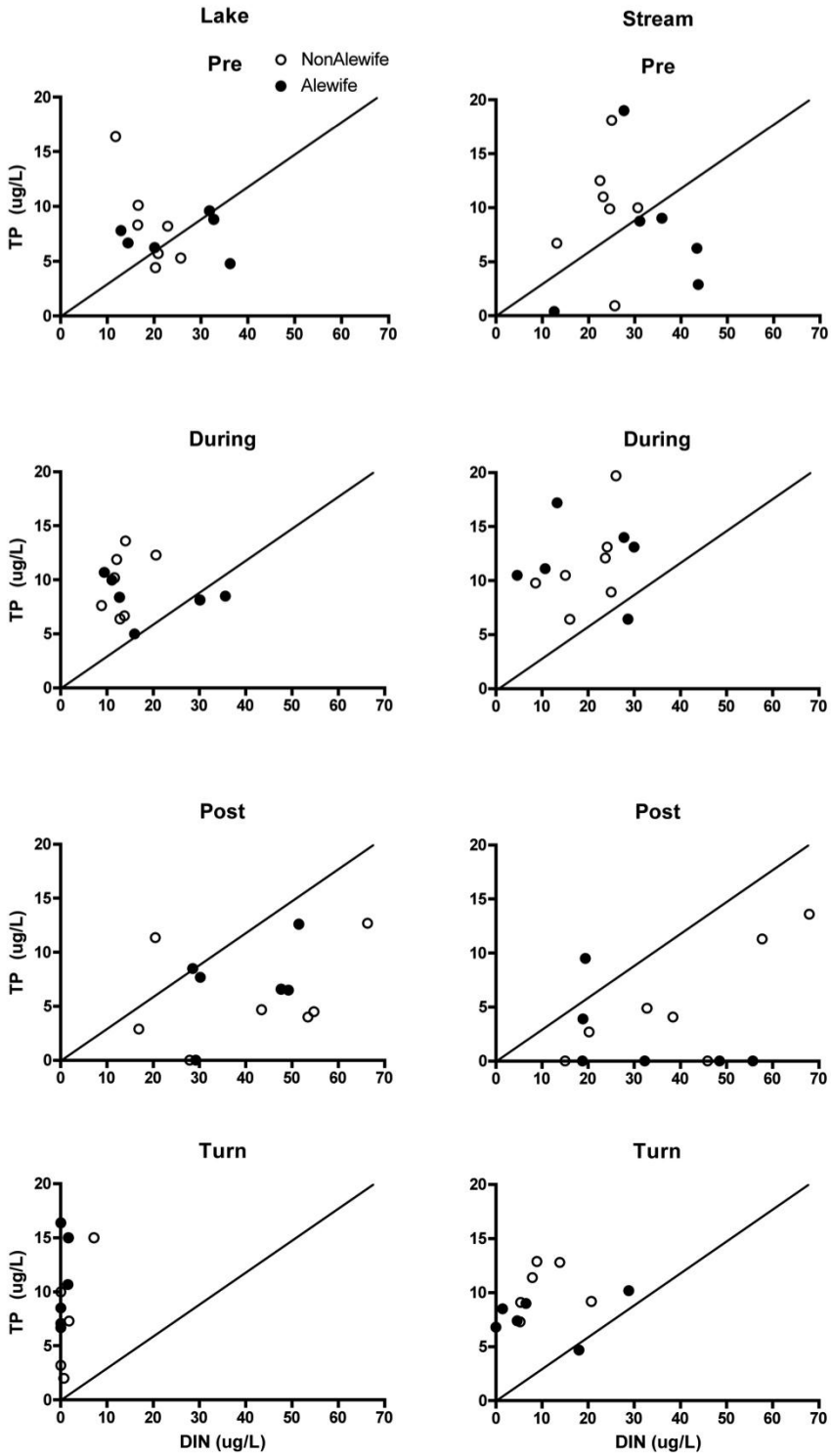
There were no significant interactions in the 2-way (fish by season) ANOVA for lake DIN (P=0.806), lake TP (P=0.709), stream DIN (P=0.312) or stream TP (P=0.984), indicating that any alewife effect on DIN or TP was not dependent upon season. Results from the Bonferroni post-hoc test show that there were significant (P<0.001) seasonal main effects in DIN for lakes and streams (Figure 7), but not in lake TP (P=0.191) or stream TP (P=0.025). There were no significant "fish" main effects (P≥0.195) between alewife and non-alewife lakes or streams.

Figure 7. DIN and TP for alewife and non-alewife lakes and streams across the four sampling seasons. Different letters above standard error bars indicate statistical significance within those alewife or non-alewife groupings.



Seasonal shifts were observed in lake and stream DIN:TP ratios (Figure 8). The diagonal line in all the graphs is representative of a DIN:TP of 3.4:1, which was proposed by Bergström (2010) as a threshold ratio indicating when phytoplankton in oligotrophic lakes switch from being N to P limited. Following this, points above the 3.4:1 line would indicate conditions favorable for N limitation and points below the line P limitation. For the Pre period there was potential N, and P limitation, which was followed by predicted N limitation in the During period. Then there was a shift to predominantly P limitation conditions in the Post period, and then a return to predicted N limitation after lake turnover.

Figure 8. DIN:TP for lakes and streams in all four sampling periods. The diagonal line indicates a DIN:TP of 3.4:1 (Begström 2010). Values above line indicate potential N limitation, and below possible P limitation. Closed circles are alewife systems and open circles are non-alewife.



2.3.2 Limiting Nutrients and Response Ratios

Nutrient limitation of primary producers was determined based on the chl*a* data and a 2-way ANOVA. Assays showed a range of responses across the four sampling periods (Table 5) including no limitation (39 lakes, 18 streams), primary N limitation (8 lakes, 25 streams), primary P limitation (3 lakes, 2 streams), co-limitation (4 lakes, 2 streams), primary N and secondary P limitation (1 lake, 2 streams), and primary P and secondary N limitation (1 stream). Streams were mostly N-limited across all seasons. Lakes were also in general N-limited, but overall had fewer instances of any limitation. Non-alewife streams had significant limitation in 70% (19/27) of occurrences compared to 56% in alewife streams (13/23). In lakes accessible to alewife, there were no consistent changes in identity of nutrient limitation from Pre to During alewife runs. Some alewife systems switched from limitation to no-limitation (Fields-lake and Toddy-stream), or co-limitation to singular limitation (Somes-stream), but some non-alewife systems also showed changes (Branch-lake, Brewer-stream). Following the large increase in DIN in August (Figure 6), it was expected that systems would shift to P- or co-limitation (Figure 8). This shift was seen in one stream, and three lakes, but not as a common trend (Table 5).

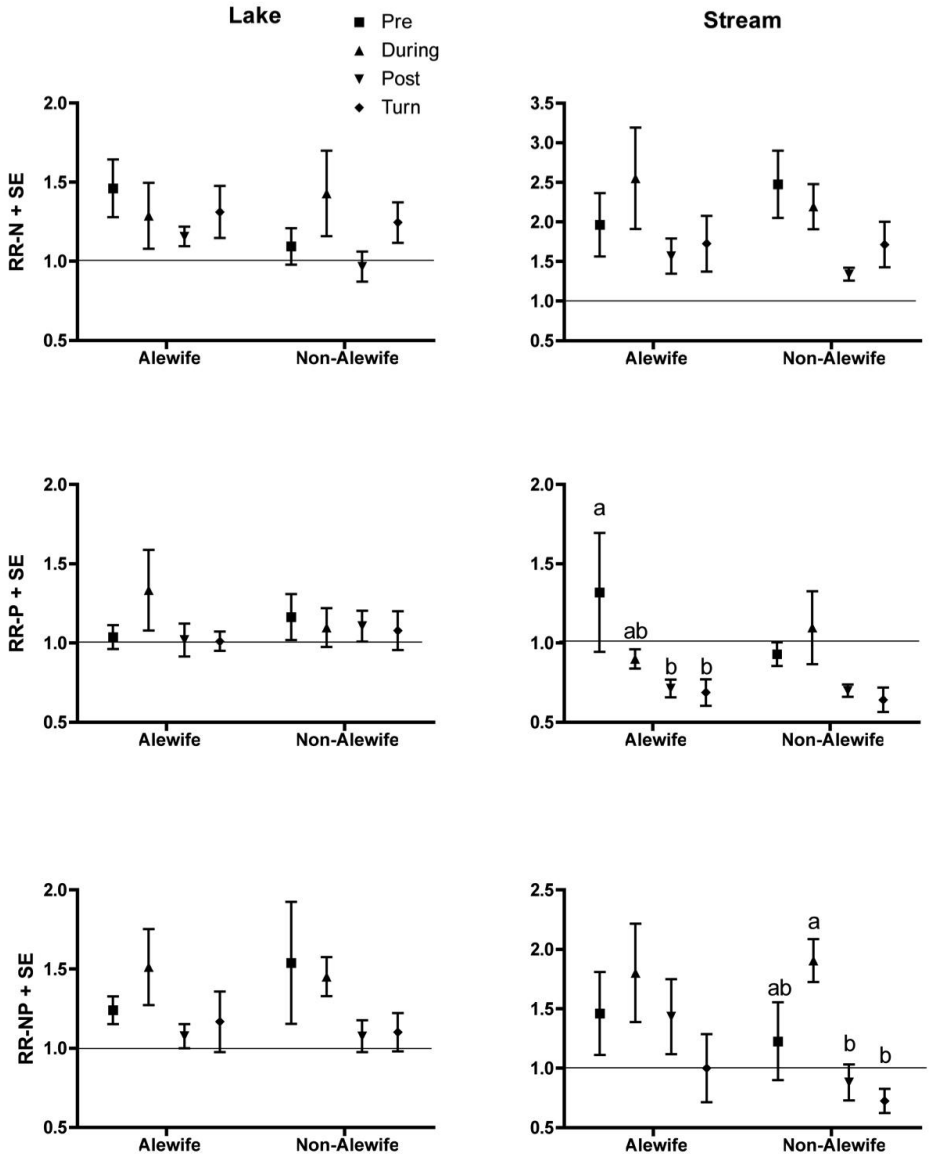
Chl*a* values from the controls of each assay, as a proxy for biomass, were used to evaluate any trends in changes to the overall standing stock of the primary producers. Results from a 2-way ANOVA (fish by season) of this control-chl*a* data revealed that there was no significant interaction ($P \geq 0.702$) and no significant main fish effects ($P \geq 0.297$) for lakes, or streams. There was only a significant main seasonal effect in streams ($P=0.046$) and not in lakes ($P=0.398$).

Table 5. Summary of lake and stream limiting nutrients. All limiting nutrients were determined based on NDS chl*a* values with two-way ANOVA. $P<0.05$ unless designated with a ~ indicating it was marginally significant ($P=0.06$). No significant limitation is denoted ---, NP means co-limitation, N, 2P means primary N secondary P limitation, P, 2N means primary P secondary N limitation and # means there was no data for that site or season. Each limiting nutrient has been color coded to assist with visual differentiation of shifts in limitation.

	Lake				**Stream**			
Site Name	Pre	During	Post	Turnover	Pre	During	Post	Turnover
Alewife								
Fields	N	---	N	---	N	N	N	---
Somes	N	N	---	~NP	NP	N	N	---
Alamoosook	---	P	N	---	#	N	N	N
SCP	---	---	---	---	---	N	N, 2P	N
Long	---	~N	---	---	---	---	---	---
Toddy	---	---	---	---	P, 2N	---	---	---
Hermon	---	---	P	---	#	#	#	#
Non-Alewife								
Davis	---	---	---	N, 2P	N	N	---	---
Swetts	---	---	~P	---	#	N	---	N
Brewer	~N	~NP	---	---	NP	N	N	N
Echo	---	N	---	---	N	P	---	---
Eagle	---	N	---	---	P	N	N	---
Branch	~NP	---	---	---	N	N	N	---
Etna	---	---	NP	---	N	N, 2P	---	N

Response ratios for all assays, treatments, and seasons are shown in Appendix A Table A.2. For all three treatments RR ranged from 0.444 to 3.687 in lakes and 0.305 to 4.750 in streams. There was no interaction between the fish (alewife and non-alewife) and season ($P\geq0.321$) lake, and stream, RR-N, RR-P, and RR-NP. There were also no significant differences for the main effect of fish (alewife and non-alewife) for lakes and streams ($P\geq0.226$). There were significant seasonal differences in stream RR-P ($P=0.012$) and RR-NP ($P=0.006$) (Figure 9). Results from the individual site 2-way (treatments by season) ANOVAs for each lake are shown in Appendix A Figure A.1., and stream in Appendix A Figure A.2.

Figure 9. Alewife and non-alewife lake and stream nutrient limitation response ratios across sampling periods. Significant differences between seasons ($P<0.01$) are marked with letters. Graphs with no letters indicate no differences in RR between any of the four seasons. Solid horizontal lines represent controls (RR=1) as a reference.



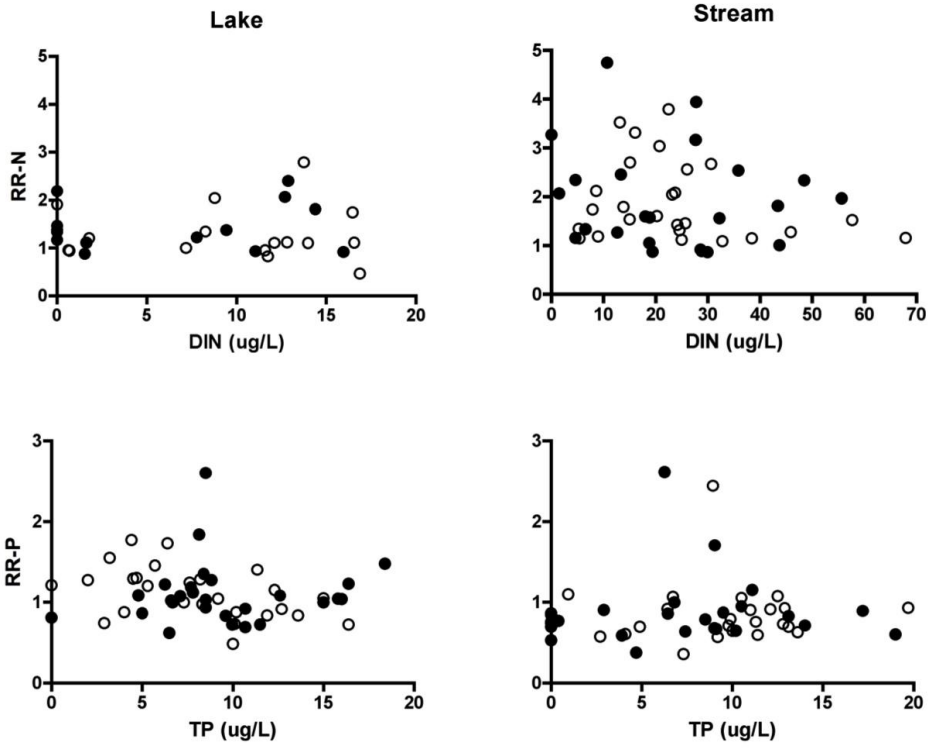
The AFDM data were analyzed in the same way as the chl*a* data to determine a rough estimate of what nutrient might be limiting the heterotrophs on the NDS (Appendix A Table A.3.) Additionally, after calculating RRs for the AFDM data, these were analyzed in the same 2-way (fish by season) ANOVA. There were no interactions for any

of the RRs ($P \geq 0.127$), no main fish effects ($P \geq 0.562$) and significant main seasonal effects ($P \leq 0.0005$).

2.3.3 Water Nutrient Concentrations and Response Ratios

Results from the linear regressions reveal that there were no significant relations between water nutrient concentrations and assay response ratios (Figure 10). For lakes there was no relation ($P=0.079$, $R^2=0.056$) between RR-N and DIN and no relation between RR-P and TP ($P=0.357$, $R^2=0.016$). Similarly for streams there was no correlation ($P=0.202$, $R^2=0.034$) between RR-N and DIN and no correlation ($P=0.785$, $R^2=0.002$) between RR-P and TP.

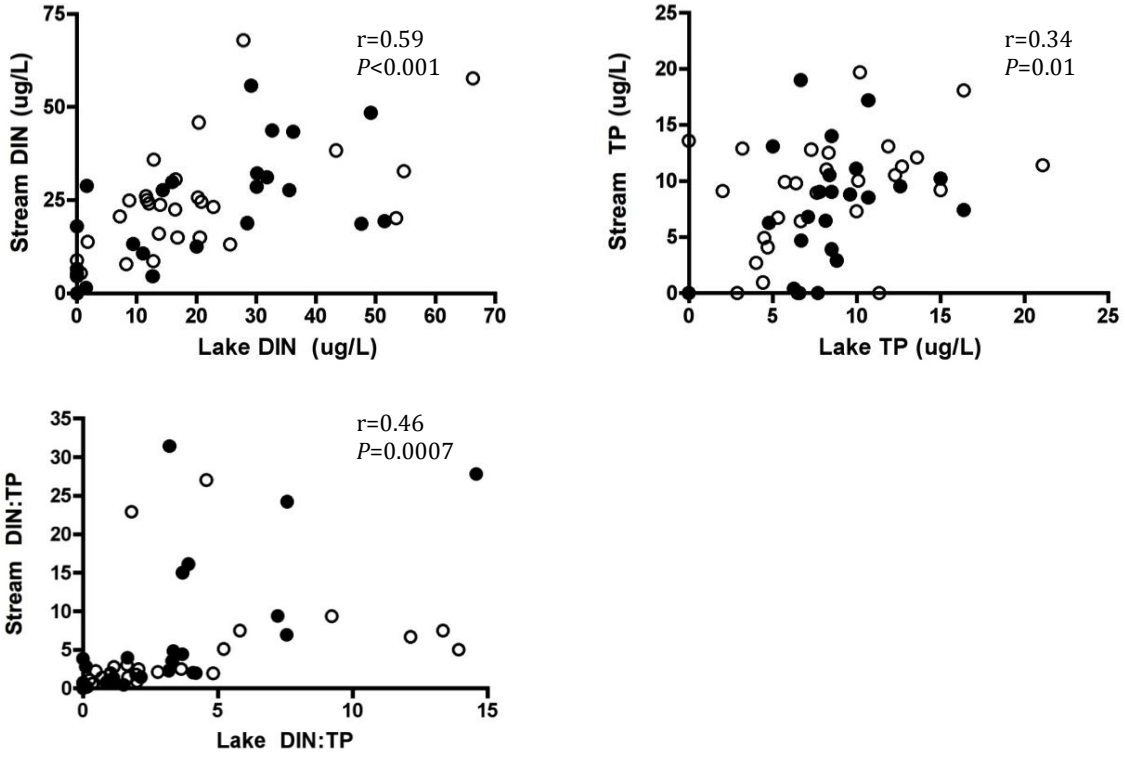
Figure 10. Nutrient concentrations vs. associated nutrient limitation response ratios. Alewife sites are solid circles and non-alewife are open circles.



2.3.4 Lake-Stream Correspondence

Correlation analysis between lake and stream nutrient concentrations revealed that DIN of lakes and streams were correlated positively (r=0.593 P<0.001). TP was correlated positively (r=0.340, P=0.014) for matched sampling periods (Figure 11). There was also correlation between the DIN:TP of lakes and streams (r=0.459 P=0.0007) (Figure 11).

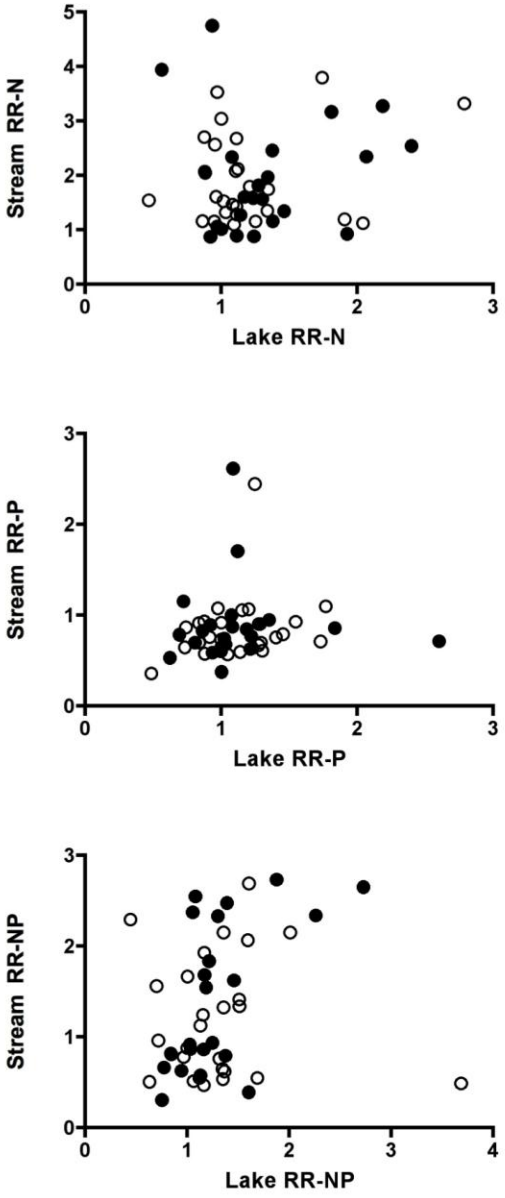
Figure 11. Correlation of lake-stream DIN, TP and DIN:TP. These graphs show matched DIN values (top left), TP values (top right) and DIN:TP (bottom). Alewife sites are solid circles and non-alewife are open circles. P and r values from Pearson's test are shown.



Based on the primary limiting nutrients determined by the assays, only five of the 13 lake-stream systems had matched limiting nutrients (or no limitation) for at least two of the four sampling periods (Table 5). Similarly, there were no significant correlations

between lake and stream RR-N (r=0.139, *P*=0.341), RR-P (r=0.095, *P*=0.515) and RR-NP (r=0.218, *P*=0.131) (Figure 12). In the majority of cases the stream had a higher RR than the lake. These differences between lake and stream RRs contrast with the findings from the nutrient concentration correlations above.

Figure 12. Correlation of lake-stream nutrient limitation RRs. Alewife sites are solid circles and non-alewife are open circles. No significant correlations were found based on Pearson's test.



2.4 Discussion

2.4.1 Effects of Alewife

The effects of MDN from alewife on lakes and streams are likely to be a consequence of demand and supply. I specifically measured the demand, or nutrient limitation status of the primary producers and found little evidence of switching or relaxation of nutrient limitation and also little change in water chemistry. The nutrients supplied by alewife have been measured previously (Durbin et al. 1979, West et al. 2010). However, the number of fish returning varies among systems and annually within systems. The results of this study suggest that there were not enough alewife returning to act as a significant ecosystem subsidy in Maine lakes and streams. Comparing fish densities from lakes used in this study to other lakes supports this hypothesis. The two highest in this study were Somes and Alamoosook with 0.03 fish/m^2 and 0.01 fish/m^2 respectively (reported here in fish/m^2 for comparison with other systems). In contrast, Bride Lake, CT had a mean density of about 0.3fish/m^2 for 2003-2008 (West et al. 2010) and Pausacaco Pond, RI had a density of 0.7fish/m^2 in 1959 (Durbin et al. 1979). These are very large differences in the density of alewife, which could be a factor in why a strong alewife effect was not found in this study.

If there had been a measurable alewife effect on nutrient limitation RRs during the spring spawning run as predicted, then in the 2-way ANOVA (fish by season) there should have been an interaction between the two factors. Since no such interaction was found, it suggests that any potential alewife effect was not dependent upon season. However, inspection of the main effects of fish from the 2-way ANOVA showed that there were no significant fish effects, and thus differences between alewife and non-

alewife systems. I had predicted that nutrient limitation would be alleviated, meaning RRs would decrease or that the identity of the limiting nutrient would change in alewife systems, but not in non-alewife systems, due to nutrients brought by migrating alewife. Ruegg et al. (2011) used this same nutrient limitation approach and they did measure an alleviation of nutrient limitation in stream periphyton by MDN brought by migrating Pacific salmon. In the sites of this study, the overall seasonal patterns of the magnitude of limitation were not as different as expected between alewife and non-alewife systems. Instead, the observed patterns of RRs were due to seasonal variations as shown by the main effects of the seasonal factor in the 2-way ANOVA. Additionally, a possible lag-release of nutrients from alewife carcasses in the fall after lake turnover did not appear to occur based on the seasonal RR data. Perhaps there were not enough carcasses/nutrients released relative to the lake sizes to elicit a response.

The stream for Somes had the highest density of alewife of the study sites (See Table 1 for densities) and did show a decreasing trend in its RRs from Pre to During to Post (Appendix A Figure A.2.), but the RRs of Pre and During were not significantly different and Somes Pond had no clear trend. For the non-alewife streams two of the seven, Eagle and Echo, had strong increases in limitation (shown by increases in the RRs) while the others did not. Characteristics of these two specific lakes could explain the increase because they are both relatively deep, clear, oligotrophic lakes with granite bedrock basins. Any nutrients would then be incorporated quickly by phytoplankton in the spring.

As Marczak et al. (2007) discussed in their meta-analysis, studies should acknowledge if a system needs a subsidy. It appears that at least one of the streams,

Long, did not exhibit any nutrient limitation for any of the seasons. This would imply that despite receiving a small number of alewife, the periphyton was not initially nutrient limited and as a result should not respond to nutrients delivered by fish. Seal Cove also was not nutrient limited for the Pre season, but then had significant increase in RR-N and became N-limited for the During period. All of the rest of the alewife streams were nutrient limited, suggesting they had the potential to respond to nutrient subsidies brought by alewife.

After the adults have returned to the ocean or died in the lakes, alewife are still influencing lakes through their offspring. Juvenile YOY are classic size selective planktivores, dictated by their gape and gill-raker size (Books and Dodson 1965, Post et al. 2008). Through this feeding behavior, YOY have been found to exert a strong trophic effect on lake zooplankton (Post et al. 2008, Demi 2010 Masters thesis). YOY grazing on zooplankton caused a distinct seasonal pattern in most alewife lakes in this study where mean zooplankton (cladoceran) body length decreased in late summer. Non-alewife lakes did not follow this same pattern. Post et al. (2008) also found that lakes with anadromous alewife had significantly higher edible chl*a* concentrations per unit spring TP compared to non-alewife lakes, suggesting decreased herbivory by zooplankton. These cascading effects on zooplankton and phytoplankton by alewife have the potential to ultimately change trophic interactions in lakes. For example, Sterner (1986) found that the indirect effect of nitrogen regeneration caused by *Daphnia pulex* had almost as large of a positive effect on the phytoplankton community as the negative effect of direct grazing. Therefore, if alewife prey on large zooplankton like *Daphnia spp.* (Post et al. 2008) then there is less grazing pressure on phytoplankton, but also a potential decrease in nutrient

recycling. Thus, the specific community composition of zooplankton and phytoplankton in individual lakes can play an important role in determining the effects of trophic changes. Trophic effects of juvenile alewife can be thought of as a secondary influence of anadromous alewife, or as a process effect on food webs (Flecker et al. 2010), rather than a direct nutrient subsidy to freshwater food webs.

2.4.2 Water Chemistry

I found little evidence of an increase in N or P concentrations in lakes or streams during alewife in-migration. Marginally higher mean DIN concentrations in alewife streams during runs compared to non-alewife streams suggest that alewife may have been a source of N to streams. However, this difference in DIN was mostly due to NO_3^- and not NH_4^+. Browder and Garman (1994) did find elevated levels of ammonium-N in streams during alewife presence compared to reference streams, but they performed higher frequency sampling including while the fish were physically moving through their site, which I did not. In contrast to Browder and Garman (1994), Walters et al. (2009) did not find a difference in stream nutrient concentrations during alewife runs, but they did observe initially higher baseline N values in alewife streams. I also did not see a difference in stream nutrient concentrations or a higher initial N or P concentrations in the alewife or non-alewife streams. Walters et al. (2009) attributed their lack of elevated dissolved nutrient levels to chemical sorption and rapid uptake by autotrophs and heterotrophs that make up stream biofilms. These processes may explain why there was only a marginally higher DIN concentration. This possible uptake by autotrophs is why I performed the NDS assays, which integrated nutrient availability over time.

2.4.3 Limiting Nutrients and Response Ratios

Nitrogen was most frequently the primary limiting nutrient for systems in this study. In comparison, a meta-analysis of nutrient limitation of primary producers in various ecosystems by Elser et al. 2007 found a balanced pattern of N and P co-limitation in stream and lake pelagic habitats. However most of the lakes in my study were not nutrient limited (39 of 56 occasions). It should be noted that the chl*a* values for most of the lake assays were very low (0.0μg/L to 4.1μg/L with the exception of Hermon in August at 15.3 μg/L) and came from low readings on the spectrophotometer, which could have affected the reliability of the calculated lake RRs. This may have been a factor in why a large number of lakes appeared to have no nutrient limitation, when DIN:TP would predict more lakes to be nutrient limited. In a study of oligotrophic lakes Bergström (2010) found that DIN:TP explained >70% of the bioassay response variation and was a better indicator of nutrient limitation compared to TN:TP, which explained <30%. Bergström (2010) also found that DIN:TP over 3.4:1 suggested a shift to P-limitation of the phytoplankton. By looking at the DIN:TP for systems in this study it is not surprising that N-limitation was common, at least initially. Pre nutrient limitation was calculated from DIN and TP nutrient concentrations as N:P, which were used to predict the limiting nutrient. Based on the DIN:TP there should have been more systems limited by nutrients and larger shifts in the primary limiting nutrient.

In a wide range of streams across the U.S. and internationally, Dodds et al. (2002) found that water column TN and TP were significantly correlated with benthic chl*a* values. In another study of streams in various ecotones Tank and Dodds (2003) also

found that chl*a* was most strongly affected by the water chemistry and inversely related to DIN:SRP. N-limitation was most frequent in their streams, which was also the case in my study streams. However, connections between periphyton and water nutrient concentrations do not necessarily translate into predictability of nutrient limitation by N:P ratios. Stelzer and Lamberti (2001) found that stream periphyton were N-limited despite high N:P and were responding to absolute amounts of N and P rather than N:P. So perhaps in low nutrient stream systems the actual amount of N or P is more important than the ratio, which could be the case in some of the oligotrophic sites in this study.

Francoeur et al. (1999) examined the nutrient limitation patterns of streams across season, reviewed results of other studies, and concluded that there is no consistent relation between stream water N and P concentrations and the nutrient limitation status of primary producers in a system. Predicting limitation patterns from N:P is more difficult in streams as there is a lower yield of chlorophyll per unit nutrient compared to lakes, possibly due to higher disturbance and different intrinsic N and P requirements (Dodds and Welch 2000). In most cases nutrient concentrations represent a snapshot of time, while limitation of autotrophs is often a more temporally-integrated metric and also depends on the intrinsic N and P demands of the community composition, and as a result water chemistry is not necessarily the best predictor of nutrient limitation.

2.4.4 Lake-Stream Correspondence

In one of the only other comparative studies of the primary limiting nutrient of lakes and their outlet streams, Marcarelli and Wurtsbaugh (2007) found that the nutrient limitation patterns in lakes and streams did not match in the their Rocky Mountain sites.

Phytoplankton of the lakes were limited primarily by N or co-limited by N and P. In contrast, the periphyton in streams was primarily P-limited. Our findings for lake-stream limitation patterns are consistent with this because the lakes in this study generally differed from outlet streams in their primary limiting nutrient and nutrient RRs. However, there was a significant correlation between the DIN concentrations of lakes and outlet streams, and weak correlation between TP concentrations. In general, lake and stream DIN:TP also showed similar seasonal shifts in the predicted limiting nutrient. Lakes may have influenced incoming water nutrient concentrations to streams sites since most were close to the lake outlet (typically <100m from the lake outlet). In examining the differences between RRs, periphyton and phytoplankton could have different intrinsic nutrient requirements (Marcarelli and Wurtsbaugh 2007) or there could be different rates of nutrient cycling occurring in each system, which could lead to variation in the identity of primary limiting nutrients.

Overall the data indicated that alewife have a variable and relatively small effect on nutrient limitation type and magnitude in the study lakes and streams. There was some evidence of an increase in N concentrations, but little evidence for a shift or alleviation of nutrient limitation in alewife systems overall relative to non-alewife systems. Additionally, lakes and streams rarely exhibited synchronized limitation patterns regardless of season or alewife presence/absence.

2.5 Conclusions

While current alewife runs may have subsidized some of the systems for some seasons, I found no distinct, consistent signature of alewife as nutrient sources across

systems in comparison to the non-alewife systems. My findings from the nutrient limitation assays did not support my original hypothesis that MDN brought by alewife would alleviate or shift nutrient limitation in lakes and streams. This may be due to small alewife run size or the role of other limiting factors among systems. Despite the results of this study, alewife do physically move from the marine to freshwater environments and several synthesis papers (Willson et al. 2004, Flecker 2010, and Lamberti et al. 2010) have acknowledged the known and potential importance of anadromous fish as ecological links between aquatic systems. The results of my study add to this larger understanding of alewife as nutrient vectors, but also reveal the variability and complexity of ecosystem nutrient subsidies. Future studies examining the effects of alewife in freshwater ecosystems should consider systems with high-density runs, and if possible, follow a system from pre- to post-reintroduction of alewife.

The findings discussed here are relevant to management of lakes and the river restoration currently underway on the Penobscot River in Maine. My results indicate that alewife are not significant nutrient subsidizers of lakes and streams, at least at the current size of alewife runs. As a result, the return of alewife during restoration in Maine oligotrophic/mesotrophic lakes may pose little threat of eutrophication, which others have also concluded (Kircheis et al. 2002). Because the majority of streams were N limited, the N portion of MDN are of more concern than the P of MDN brought by current or future alewife. Additionally, the YOY exerted potentially important trophic pressures on lake food webs, with consequences for zooplankton and phytoplankton.

As I predicted based on findings from other studies, this study found that lakes and outlet streams rarely followed the same limitation patterns. Lakes and streams did

exhibit correlation between their water column nutrient concentrations though, and the pathways and processes that influence lake water chemistry could have downstream impacts on outlet streams. Examining these patterns is important because connectivity between ecosystems and a landscape scale perspective are becoming increasingly important in our understanding of ecosystem dynamics, resiliency, and restoration. Interdisciplinary and inter-habitat approaches then are necessary to further understanding of linked, complex, open boundary ecological systems.

Chapter 3

STABLE ISOTOPES: TRACKING MDN UP THE FOOD WEB

3.1 Introduction

Stable isotopes of common elements (^{13}C, ^{15}N, $^{34}Sulfur$, $^{18}Oxygen$, $^{2}Deuterium$) have been used as natural tracers to track trophic positions of organisms, or the origins of a consumers diet (Layman et al. 2011). The trophic position of an organism in a food web can be estimated by using the ^{15}N isotope because N fractionates predictably with trophic transfers (Layman et al. 2011). Thus, predators are more enriched in ^{15}N compared to their prey, and ~3.4 $\delta^{15}N$ per trophic level can be used to calculate food chain length (Post 2002). ^{13}C and ^{34}S are different from ^{15}N in that they do not change much with trophic level, but concentrations vary among primary producers, which means ^{13}C and ^{34}S can be indicators of the origin of dietary C or S. For example, in lakes the littoral habitat is commonly more enriched in ^{13}C than the pelagic habitat and so stable isotope analysis of ^{13}C can help differentiate between these energy/nutrient sources (France 1995, Post 2002). The marine environment is enriched in both ^{13}C and ^{34}S, and to a lesser extent ^{15}N, compared to freshwater ecosystems and so these isotopes have been used to differentiate between marine and freshwater nutrients and organisms (Layman et al. 2011). This approach has been used to understand the influence of anadromous fish on freshwater systems.

Through stable isotope analysis, many studies have used the unique marine characteristic of alewife and salmon nutrients to track where the nutrients from these fish go once in freshwater systems. Because adult alewife spend 3-5 years accumulating most

of their biomass in the ocean, they have a distinct marine isotope signature when they return to freshwater systems. Stable isotope ratios of carbon, nitrogen, and sulfur (^{13}C, ^{15}N, and ^{34}S) have been used broadly to understand energy flow, trophic food chain structures, and nutrient dynamics in aquatic systems.

Several studies have used stable isotopes to track the enrichment of lake and stream organisms at different trophic levels following spring alewife runs (Garman and Macko 1998, MacAvoy 2009, Walters et al. 2009, and Hanson et al. 2010). Garman and Macko (1998) used stable isotopes to examine alewife subsidies in Atlantic coastal freshwater systems, specifically Wards Creek, Virginia. The only food chain pathway to derive a substantial portion of its biomass from MDN was direct consumption of alewife by piscivores (Garman and Macko 1998). Similarly, MacAvoy et al. (2000) found that in the Rappahannock River, Virginia, the alewife spawning run provided an important source of nutrients to predatory fish. In a recent study by Hanson et al. (2010), elevated $\delta^{13}C$ levels were found in the freshwater amphipod *Gammarus fasciatus* during the spawning run, relative to pre and post-run levels, indicating that alewife provided MDN to the freshwater system, which were then incorporated into stream invertebrates.

Walters et al. (2009) also employed stable isotopes to examine the incorporation of MDN from alewife into stream food webs. Their isotope sampling encompassed before, during, and after spring alewife runs. They found that relative to non-alewife streams $\delta^{15}N$ values were higher, usually at least double, in the alewife stream organisms. Periphyton $\delta^{15}N$ peaked just after the alewife run, followed by collector-gatherer insects and then predatory insects, but a $\delta^{13}C$ shift was only found in periphyton (Walters et al. 2009). This increase in ^{15}N demonstrated that stream organisms use some MDN from

alewife and the lag-effect in ^{15}N peaks points towards nitrogen incorporation at lower trophic levels first before moving up the food chain (Walters et al. 2009).

In a study of Pacific salmon, Chaloner et al. (2002) used C and N stable isotopes to track the food web incorporation of salmon MDN in both natural and artificial (stocked with salmon carcasses) streams. They found that all the trophic levels measured (biofilms, aquatic macroinvertebrate detritivores, shredders and predators, coho salmon and cutthroat trout) incorporated marine-derived nitrogen and carbon, but chironomid midges assimilated relatively more MDN than others (Chaloner et al. 2002). Their results exemplified the potential ecological importance of salmon MDN to freshwater systems in southeastern Alaska (Chaloner et al. 2002).

Some evidence exists then to show that MDN are incorporated into stream food webs, but little research has been done to connect this with the potential importance of an alewife subsidy. Overall, many studies (Garman and Macko 1998, Hanson et al. 2010, MacAvoy et al. 2009, West et al. 2010) acknowledged that the amount of MDN brought to the freshwater systems, and thus its potential importance, depended on the size of the alewife runs, which have in general declined over the past decades. The use of stable isotopes to detect/track MDN also has great value as a management tool. In particular, it is being use to study dam removal and the effectiveness of restoring diadromous fish in the Penobscot River, Maine (K. Wilson, personal communication).

I used stable isotope analysis to track MDN brought by alewife to lakes. To determine if nutrients brought to the freshwater systems by alewife were incorporated into the lake food web, I measured and compared the stable isotope signatures of different lake trophic levels. Three lakes were chosen for comparison and trophic

components analyzed included lake zooplankton, resident fish and juvenile and adult alewife. Overall this survey of lake organisms aimed to detect a marine isotope signal in freshwater fish from MDN brought by alewife, or a food web trophic shift of resident fish due to competition with juvenile alewife. I also wanted to test the feasibility of the isotope method in the context of anadromous alewife in Maine. I hypothesized that marine nutrients brought by alewife to freshwater systems would be incorporated into lake food webs and that zooplankton and fish would be enriched in ^{15}N and ^{13}C compared to organisms in non-alewife lakes, and fish feeding on adult alewife would be enriched in ^{34}S. This could occur through consumption of lower trophic levels that had assimilated MDN, consumption of alewife eggs, or direct consumption of alewife or carcasses. Additionally, if juvenile alewife were competing with resident fish for food resources then a shift in trophic level could be seen in those resident fish based on ^{15}N values.

3.2 Methods

Specimens for stable isotope analysis (^{15}N, ^{13}C, and ^{15}S) were collected from three to five of the lake study sites described in Chapter 2. Alamoosook Lake and Toddy Pond were the lakes receiving alewife, Swetts Pond and Brewer Lake were the non-alewife lakes, and Fields did not receive alewife in 2010, but did in 2011 after a dam removal and restoration project. In 2010, bi-weekly zooplankton samples for April-October from all five lakes were collected using a large plankton net (30cm diameter, 243μm mesh), filtered and dried. All samples for ^{13}C and ^{15}N stable isotope analysis were then pulverized, weighed on a balance, packaged in aluminum, and shipped for analysis using an isotope ratio mass spectrometer (UC Davis Stable Isotope Facility). Planktivorous,

insectivorous, and piscivorous fish from the lakes (smallmouth bass, largemouth bass, white perch, yellow perch, redbreast sunfish, black crappie, golden shiner, chain pickerel, and juvenile alewife), were collected in 2010 and 2011 by angling, beach-seine nets or trap nets in late summer/early fall (August-October), and frozen until tissue processing. This included removing a dorsal muscle tissue sample with a knife, drying it in a 60°C drying oven for at least two days, grinding the dried samples with a glass rod, and then weighing and packaging them for shipment to be analyzed for ^{13}C and ^{15}N (UC Davis Stable Isotope Facility). Separately packaged replicates of the same tissue samples were sent to be analyzed for ^{34}S (Washington State U Stable Isotope Laboratory). Tissue from snails (littoral) and mussels (pelagic) were used as a baseline of the food web (Post 2002), and migrating adult alewife from the Penobscot river estuary (from NOAA sampling) were used as a marine reference signal. These snail, mussel and alewife samples were processed in the same manner as the fish samples described above, but snail and mussel shells were removed, and only the foot of the mussel was used for analysis.

The relative enrichment of samples was calculated compared to a known reference using the following equation:

$$\delta^{13}C(‰)=((^{13}C/^{12}C_{sample})/(^{13}C/^{12}C_{standard})-1)x1000$$

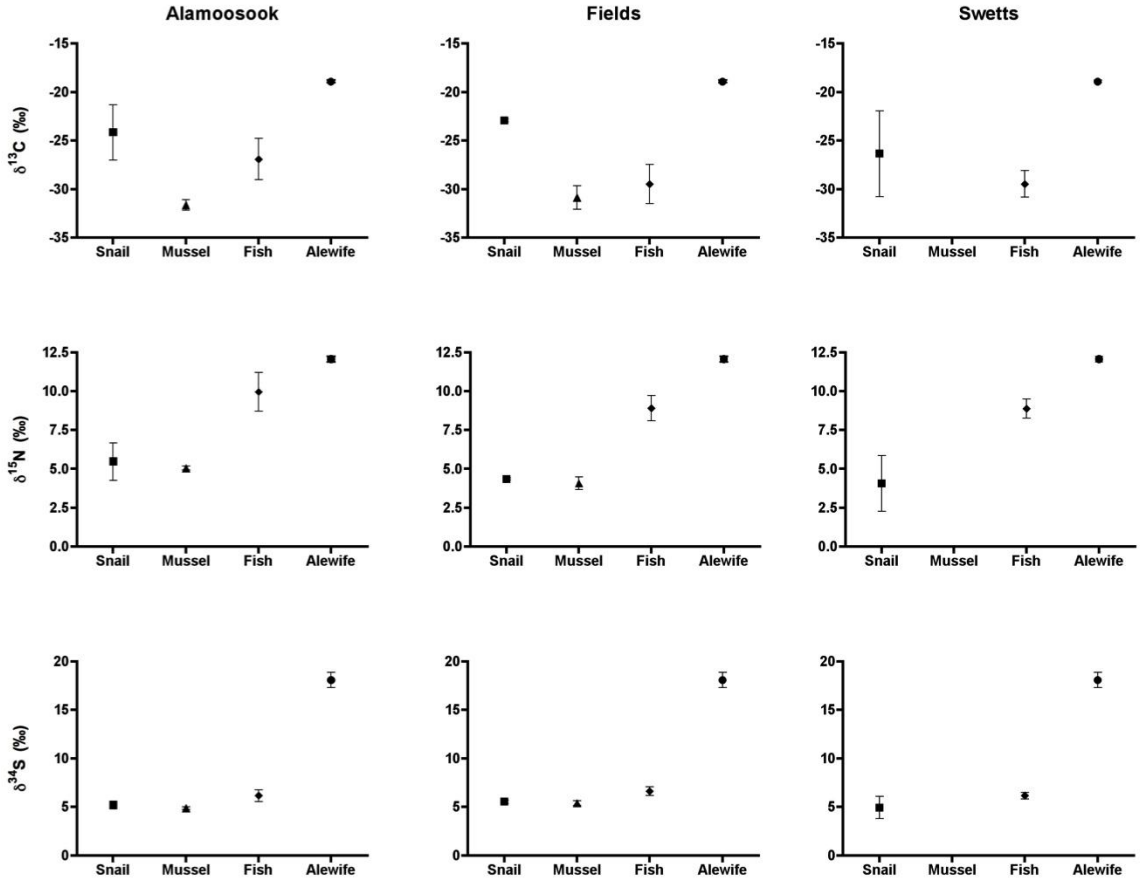
Delta (δ) values for ^{15}N and ^{34}S were calculated in the same manner. Zooplankton isotope data were plotted through time to examine seasonal changes and any enrichment due to alewife influx during spawning. The spawning window was determined by the characteristic seasonal range based on 2011 spawning days. Fish tissue isotope data were analyzed using $\delta^{13}C$: $\delta^{15}N$ bi-plots, and $\delta^{34}S$ plots. This was done to see if any fish values

were enriched towards an alewife $\delta^{13}C$, $\delta^{15}N$, or $\delta^{34}S$ value. I also calculated food chain enrichment by subtracting $\delta^{15}N$ values of fish by mean $\delta^{15}N$ baseline of snails and mussels from the specific lake. As this was a survey of lake organisms, with limited replication, I used only qualitative analysis of the data.

3.3 Results

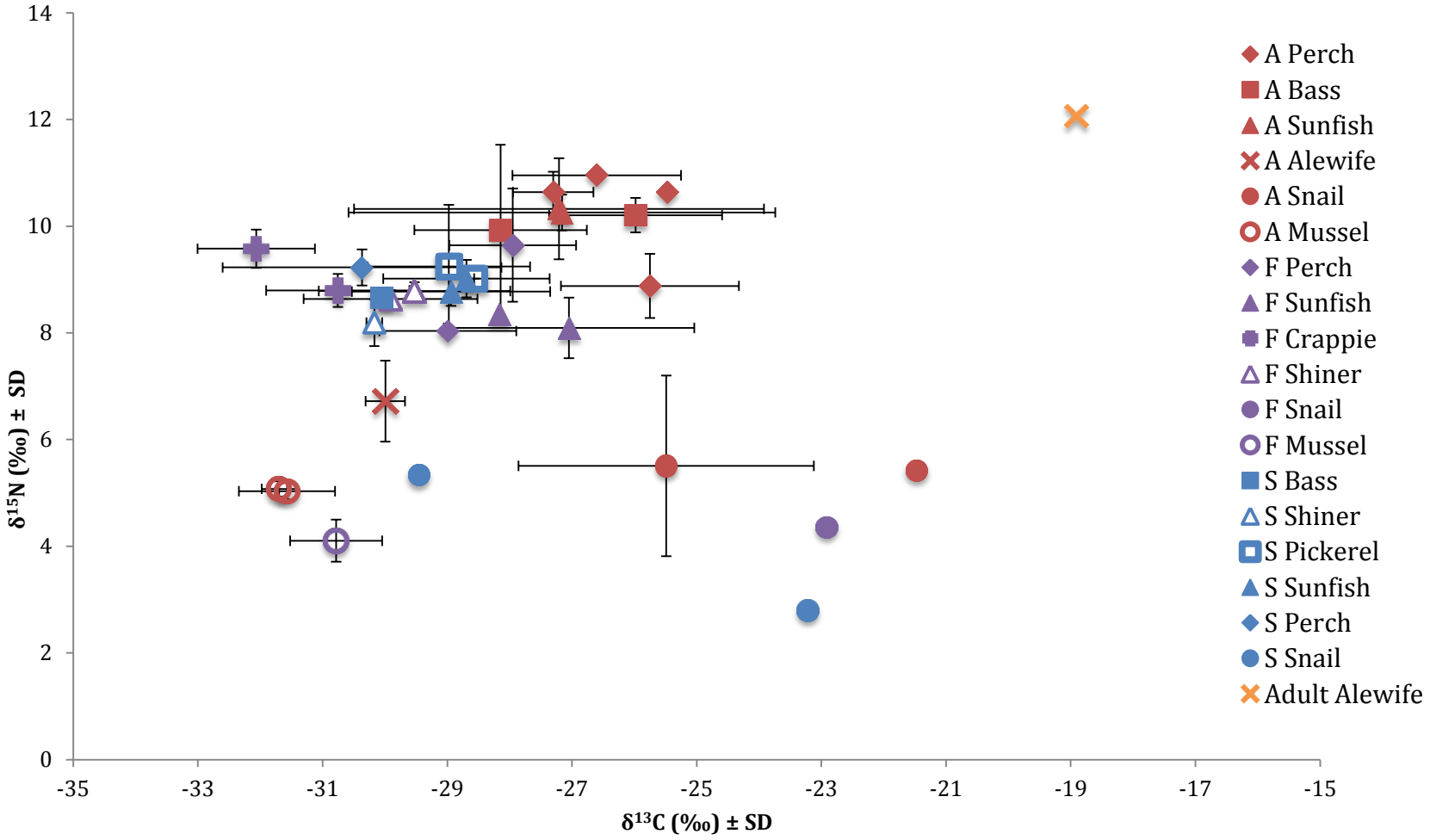
In the lakes, the snails were more enriched in ^{13}C than the mussels and resident fish (Figure 13). Fish from Alamoosook had overall higher $\delta^{13}C$ than Fields and Swetts resident fish (Figure 13). For all lakes, snails and mussels had similar $\delta^{15}N$ and resident fish were more enriched in ^{15}N than these snails and mussels. The ^{34}S data show that snails, mussels and resident fish all had similar $\delta^{34}S$ and that, by comparison, adult alewife were much more enriched (Figure 13). Thus, there was no large enrichment of any fish by ^{34}S in any of the lakes compared to the anadromous alewife.

Figure 13. Mean isotope values of lake snails, mussels, and resident fish. The values include samples from both 2010 and 2011 (±SD). Alewife values are from adults caught in the Penobscot River estuary.



Overall the Alamoosook fish appeared to be more enriched in ^{13}C and ^{15}N compared to the fish from Swetts and Fields, which was expected (Figure 14). One Alamoosook snail in particular appeared to be shifted towards the anadromous alewife signature compared to the other snail samples (Figure 14). Additionally, the littoral (snail) primary consumers were enriched by ~5‰ $\delta^{13}C$ compared to the pelagic (mussel) primary consumers.

Figure 14. Fish, snail, and mussel $\delta^{13}C$ and $\delta^{15}N$ bi-plot. Data from both 2010 and 2011 are included. Fish from Alamoosook are shown in red symbols and designated with an A in the legend. Fish from Fields are shown in purple symbols and designated with an F in the legend. Fish from Swetts are shown in blue symbols and designated with an S in the legend. Each different type of fish is with a unique style of symbol. Yellow and white perch were combined. The alewife symbol in Alamoosook Lake is for juvenile alewife, not adults.


14
12
10
8
6
4
2
0
-35
-33
-31
-29
-27
-25
-23
-21
-19
-17
-15
δ15N (‰) ± SD
δ13C (‰) ± SD
A Perch
A Bass
A Sunfish
A Alewife
A Snail
A Mussel
F Perch
F Sunfish
F Crappie
F Shiner
F Snail
F Mussel
S Bass
S Shiner
S Pickerel
S Sunfish
S Perch
S Snail
Adult Alewife

I found no evidence of a large shift in isotopic signature in Fields Pond fish from 2010 to 2011 when alewife were restored (Figure 15). White perch showed a difference between the two years as they were enriched in ^{15}N and there was also more variability in the range of ^{15}N across the individuals sampled. This ^{15}N enrichment was confirmed when looking at the values in Table 6, which show the fish compared to the baseline of primary consumers (snails and mussels).

Figure 15. Fish isotope data for Fields in 2010 and 2011. Values for 2010 are green and are orange for 2010.

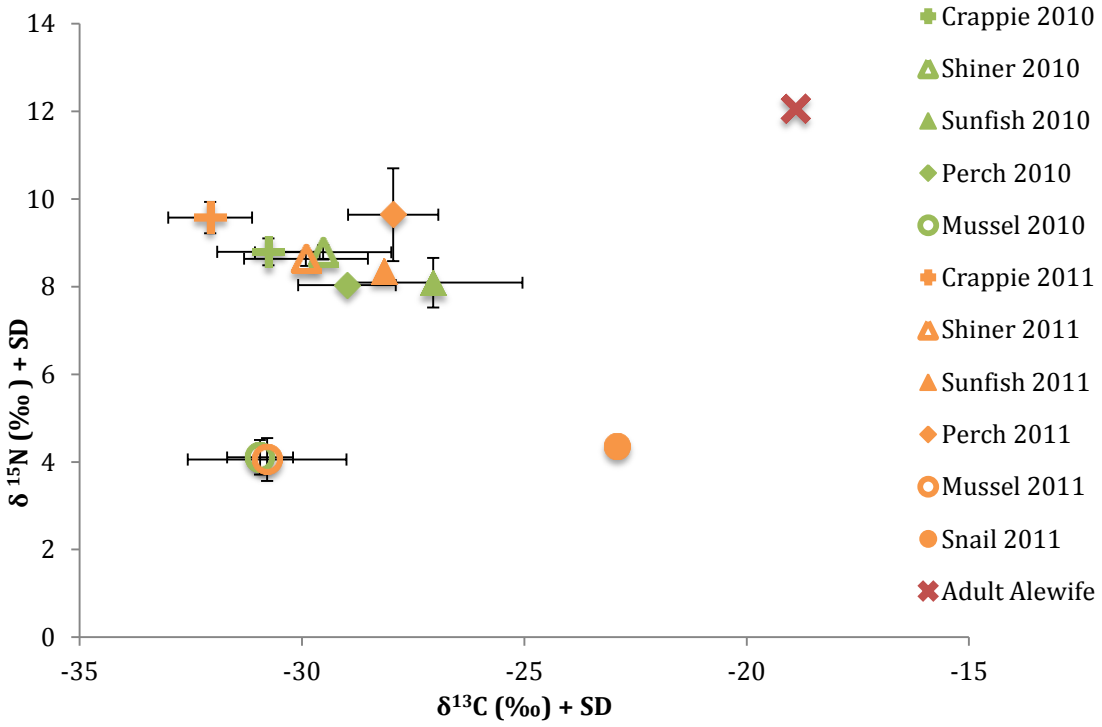


Table 6. Relative ^{15}N enrichment of individual resident fishes. All values were calculated by subtracting the measured $\delta^{15}N$ value from an average ^{15}N baseline (mean of snails and mussels). The baseline for Swetts 2010 included the zooplankton because there was only one snail value and it was comparatively low. Highlighted values were more enriched in ^{15}N compared to 2010 for the white perch, or the baseline for the zooplankton.

Fish	2010			2011		
	Alamoosook	Fields	Swetts	Alamoosook	Fields	Swetts
Sunfish	5.57	4.25	4.83	5.45	4.17	3.43
	4.33	3.34	4.31	4.78		3.93
	6.44	4.38	4.67	5.17		
	4.66					
Yellow Perch	5.12			3.68		
	5.99			3.82		
	5.75					
	5.38					
Bass	3.72		4.49	5.42		
	5.98			5.04		
				4.78		
White Perch	5.88	3.95	5.33	5.86	4.29	
		3.94	4.68	5.85	6.33	
		3.90	5.16	4.82	5.04	
					6.82	
					4.86	
Black Crappie		4.72			5.36	
		4.98			5.43	
		4.37			5.98	
					5.23	
					5.02	
Golden Shiner		4.77	3.66		4.35	
		4.49	4.51		4.58	
		4.79	3.89			
Pickerel			5.78			3.68
			5.69			
			3.74			
Zooplankton	2.55	0.86				

Generally, lake zooplankton decreased in $\delta^{15}N$ from spring to summer and then increased from late summer to fall (Figure 16). The exception to this was a spike in the $\delta^{15}N$ value of the zooplankton in Alamoosook Lake during adult alewife presence and spawning (Figure 16). The other alewife lake, Toddy, did not show any spike and instead

followed the pattern of non-alewife lake zooplankton $\delta^{15}N$. The $\delta^{13}C$ values did not show any distinct differences between alewife and non-alewife lakes (Figure 17). There was an overall increasing trend in $\delta^{13}C$ from spring to summer followed by a decline in late summer/fall.

Figure 16. Zooplankton $\delta^{15}N$ values for 2010. The grey band represents the period of adult alewife presence in the lakes. The solid symbols are the two alewife lakes and the open symbols are the three non-alewife lakes. Toddy and Alamoosook did not have zooplankton values in late summer and fall because there was not enough zooplankton material collected to process for isotopes, possibly due to predation by juvenile alewife.

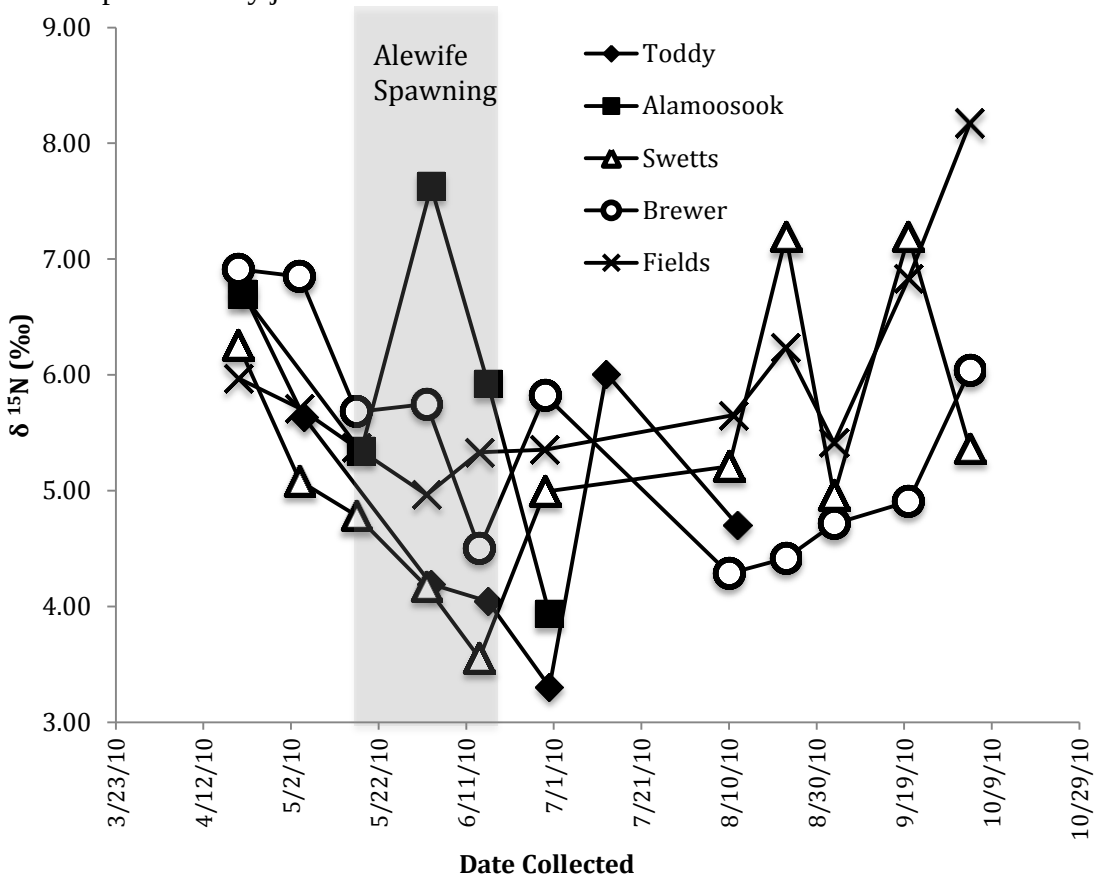
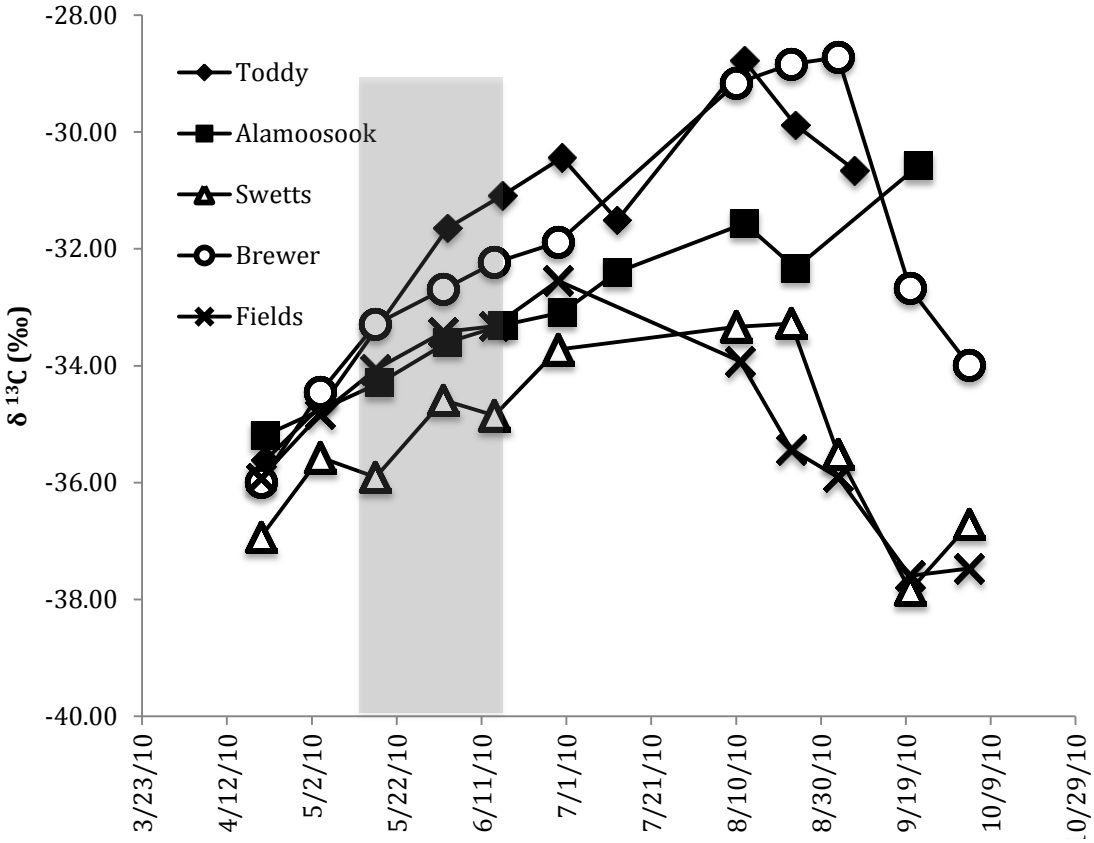


Figure 17. Zooplankton $\delta^{13}C$ values for 2010. The grey band represents the period of adult alewife presence in the lakes. The solid symbols are the two alewife lakes and the open symbols are the three non-alewife lakes.



3.4 Discussion

Adult alewife were enriched in ^{13}C, ^{15}N, and ^{34}S compared to freshwater organisms as was expected (MacAvoy et al. 2009). These enrichments showed the marine origin of the majority of their biomass. In contrast, there was little evidence of a MDN subsidy to littoral (snails) or pelagic (mussels) organisms, as overall these were not enriched in the alewife lake compared to the non-alewife lake. There was one enriched snail in Alamoosook, which could indicate some bottom-up transfer of MDN. I did find a distinct separation between littoral and pelagic primary consumers, which was expected based on previous studies of lake food web baselines (France 1995, Post 2002). Based on

the ^{15}N data, which can serve as an indicator of trophic position, I expected to observe a large separation between the trophic positions of resident fishes that should theoretically be at different food web levels. A clear separation of piscivorous and planktivorous fish was not observed in the data and one explanation could be that even the piscivorous chain pickerel were not large enough to eat adult alewife due to gape size limitation.

Garman and Macko (1998) and MacAvoy (2000) found that ^{34}S is enriched when predators eat adult alewife, but not in any other resident fish. This indicates that ^{34}S can be used to track direct consumption of adult alewife but not necessarily bottom up trophic transfer of MDN. Because there was no enrichment of ^{34}S in resident fish, the data suggest that there was no direct consumption of adult alewife. It is possible, especially based on the length/weight data (total length range of resident fish: 52mm-440mm), that few, if any, of the resident fish sampled were large enough to eat adult alewife.

The observed and calculated $\delta^{15}N$ and $\delta^{13}C$ enrichment of white perch in Fields 2011 relative to the baseline and to 2010 white perch was noteworthy. While few (~200) adult alewife entered Fields in 2011, they are prolific spawners and thousands of juveniles were seen leaving in the fall (Rob Hogg, personal communication). This means that post-spawning, Fields may have been rich with alewife eggs. White perch are known to feed on eggs of other fish so it makes sense that there would be a ^{15}N enrichment of resident fish consuming the alewife eggs. Juvenile white perch do graze on zooplankton, so they could have been competing with YOY juvenile alewife for zooplankton and in response shifted to trophically higher foods. This scenario is less likely because the white perch from Fields were adults. A more explicit exploration of this ^{15}N trend would need to be pursued to truly tease apart the specific drivers of the observed shift, especially

since white perch may naturally have a wide range of $\delta^{13}C$ values (Quenton Tuckett, personal communication).

Resident fish of Alamoosook were enriched in ^{13}C and ^{15}N compared to those in the non-alewife lake, Swetts. Zooplankton in Alamoosook were also enriched in ^{15}N as shown by the spike during spring spawning. Enrichment of Alamoosook organisms can be explained by the higher density of alewife runs in Alamoosook (Table 1). Alamoosook is downstream of Toddy so it receives more fish because fewer continue upstream and into Toddy. The zooplankton in Alamoosook were also enriched compared to the baseline of snails/mussels of Alamoosook (Table 6), although this was only a temporary enrichment based on the spike and subsequent decline in Figure 16. Based on these results, Alamoosook is most likely receiving some measureable amount of MDN from anadromous alewife, which are being incorporated into snails, zooplankton, and some resident fish.

3.5 Conclusions

I found limited evidence of a marine signal of ^{13}C and ^{15}N enrichment in Alamoosook fish and a ^{15}N enrichment in Alamoosook zooplankton. There was also a shift in the $\delta^{15}N$ of Fields white perch in 2011 after alewife reintroduction, compared to white perch in Fields in 2010. One explanation for this shift would be consumption of ^{15}N rich MDN in the form of alewife eggs. Future sampling should include larger, piscivorous fish that could potentially eat adult alewife. Then a more distinct MDN stable isotope signal may be observed in the resident fish, which has been shown for other large fish in the main-stem of the Penobscot river (Wilson, unpublished data). A study such as

this aiming to track or detect the presence of MDN in freshwater systems could also benefit from a pre/post alewife sampling design. Even from a somewhat small sample size of fish I was still able to find a MDN stable isotope signal in the alewife lake. With this in mind, the use of stable isotopes could be a very effective management or monitoring tool to track MDN into freshwater food webs, especially in the context of diadromous fish restoration.

Chapter 4

SYNTHESIS

In the process of migrating from the ocean to freshwater lakes and streams alewife have the potential to transport marine nutrients and act as nutrient subsidies to freshwater systems. In this study, I used two approaches to examine the possible subsidy effect of alewife in Maine lakes and streams: nutrient limitation assays and stable isotope analysis. The first approach measured the nutrient limitation of primary producers in lakes and streams across seasons to examine the potential for MDN to shift or alleviate nutrient limitation. I found that at the current size of alewife runs in the study sites used alewife did not have a strong, consistent effect on the nutrient limitation of lakes or streams. The value of this empirical approach was that it directly tested if alewife nutrients affected lake and stream ecosystem processes.

However, the first approach did not allow me to examine if, and where, MDN were incorporated into freshwater food webs. In this regard, the second approach using stable isotopes complements the first approach as natural tracers in food webs. I found a clearly distinct signature in alewife C, N and S isotopes suggesting that stable isotopes are a potentially useful tool in identifying an alewife nutrient subsidy to Maine lakes. However, in my survey I found limited evidence of enrichment in ^{13}C and ^{15}N of alewife lake zooplankton and fishes. This suggests that MDN brought by adult alewife were incorporated into some food web compartments, but consistent with evidence from my nutrient limitation assays, current alewife nutrient delivery may be small. Together these two approaches provide a more complete picture of the influence of alewife MDN in freshwater ecosystems. Without one or the other it would be even more difficult to tease

apart the inherently complex and variable role of MDN in freshwater nutrient limitation and food webs.

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APPENDIX A
SUPPLEMENTARY TABLES AND FIGURES

Table A. 1. Secchi disc depths. Values with at * mean that the secchi disc hit bottom there, and --- means there was no secchi value for that date due to high winds.

	May	June	Aug	Oct
Alewife				
Seal Cove	4.2	4.4	4.6	5.4
Long	4.3	6.8	6.9	7.6
Somes	3.5	4.4	4	3.9
Fields	3.5	3.4	3.3	3.6
Alamoosook	3.5	4.2	4.8	4
Toddy	3.8	4	4.6	4.9
Hermon	2.5	2.4	2.6	---
Mean	3.6	4.2	4.4	4.9
Non-Alewife				
Echo	6.7	7	6.2	8.3
Eagle	5.5*	9.9	10.5	10.9
Brewer	4.3	4.2	4.2	5.8
Swetts	3.4	3.9	4.6	3.6
Branch	5.7	---	8.7*	8.4
Etna	2.5	2.5	3.4	2.3
Davis	2.8	3.7	4.3	3.9
Mean	4.4	5.2	6.0	6.2

Table A. 2. Lake and stream response ratios from the nutrient limitation assays. Response ratios are listed for the N treatment (RR-N), P treatment (RR-P) and NP treatment (RR-NP). N/A means there was no data for that site or season. See methods for RR equation.

		Lakes				Streams			
	RR	Pre	During	Post	Turnover	Pre	During	Post	Turnover
Alewife									
Alamoosook	RR-N	1.3	0.6	1.3	1.5	N/A	3.9	1.9	1.3
	RR-P	0.8	2.6	0.8	1.0	N/A	0.7	0.7	0.7
	RR-NP	N/A	2.7	1.3	1.0	N/A	2.7	2.3	0.9
Somes	RR-N	2.4	2.2	1.3	0.9	2.5	2.3	1.6	2.1
	RR-P	1.1	1.4	1.2	0.7	1.7	1.0	0.8	0.8
	RR-NP	1.4	1.9	1.2	1.0	2.5	2.7	1.5	0.9
SCP	RR-N	1.1	0.9	1.1	2.2	1.3	4.8	2.3	3.3
	RR-P	1.2	0.7	0.6	1.1	0.8	1.2	0.5	1.0
	RR-NP	1.1	1.1	1.1	2.3	0.6	2.5	2.4	2.3
Long	RR-N	1.0	1.9	1.0	1.4	1.0	0.9	1.1	1.2
	RR-P	1.3	1.8	1.0	1.2	0.9	0.9	0.7	0.6
	RR-NP	1.4	1.6	0.8	1.2	0.8	0.4	0.8	0.9
Toddy	RR-N	1.3	0.9	1.2	1.2	1.8	0.9	0.9	1.6
	RR-P	1.1	0.9	1.1	1.0	2.6	0.8	0.9	0.4
	RR-NP	1.2	0.8	1.2	0.9	1.8	0.8	0.9	0.6
Fields	RR-N	1.8	1.4	1.2	1.1	3.2	2.5	1.6	0.9
	RR-P	1.0	0.9	0.9	1.0	0.6	0.9	0.6	0.6
	RR-NP	1.5	1.2	0.8	0.8	1.6	1.7	0.7	0.3
Hermon	RR-N	1.3	1.2	0.9	1.0	N/A	N/A	N/A	N/A
	RR-P	0.7	1.0	1.5	1.0	N/A	N/A	N/A	N/A
	RR-NP	0.9	1.3	1.2	0.9	N/A	N/A	N/A	N/A
Non-Alewife									
Branch	RR-N	1.0	1.1	0.5	1.0	3.5	2.1	1.5	N/A
	RR-P	1.2	1.7	0.7	1.0	1.1	0.7	0.9	N/A
	RR-NP	1.6	1.5	0.7	1.2	2.1	1.3	1.6	N/A
Etna	RR-N	0.9	1.1	1.3	1.0	2.1	1.4	1.2	3.0
	RR-P	1.3	0.8	1.3	1.0	0.9	0.7	0.6	0.6
	RR-NP	1.4	1.0	1.1	1.1	1.3	1.7	0.6	1.1
Eagle	RR-N	1.1	2.8	1.0	1.3	1.5	3.3	1.6	1.4
	RR-P	1.8	1.0	0.9	0.5	1.1	0.9	0.6	0.4
	RR-NP	3.7	1.6	0.7	0.6	0.5	2.7	1.0	0.5
Echo	RR-N	1.0	2.0	1.1	0.9	1.3	1.1	1.1	1.2
	RR-P	1.5	1.2	1.3	1.3	0.8	2.4	0.7	0.7
	RR-NP	1.4	2.0	1.2	1.1	0.5	2.2	0.5	0.5
Davis	RR-N	1.1	1.1	0.9	1.9	2.7	2.1	1.2	1.2
	RR-P	0.7	0.8	1.2	1.6	0.6	0.9	0.6	0.9
	RR-NP	0.4	1.2	1.3	1.7	2.3	1.9	0.8	0.5
Brewer	RR-N	1.7	1.0	1.0	1.2	3.8	2.6	1.5	1.8
	RR-P	1.0	0.9	0.9	1.0	1.1	0.9	0.8	0.7
	RR-NP	1.3	1.5	1.2	1.0	0.7	1.4	1.2	0.9
Swetts	RR-N	0.8	0.9	1.1	1.3	N/A	2.7	1.3	1.7
	RR-P	0.7	1.2	1.4	1.1	N/A	1.1	0.8	0.6
	RR-NP	1.0	1.4	1.4	1.0	N/A	2.2	0.6	0.8

Figure A. 1. Individual lake ‘treatment by season’ results for RRs. Significant differences between seasons for each individual treatment (P<0.05) are marked with letters. Treatments with no letters indicate no differences in RR between any of the four seasons. Solid horizontal lines represent no change in treatment when compared with controls (RR=1).

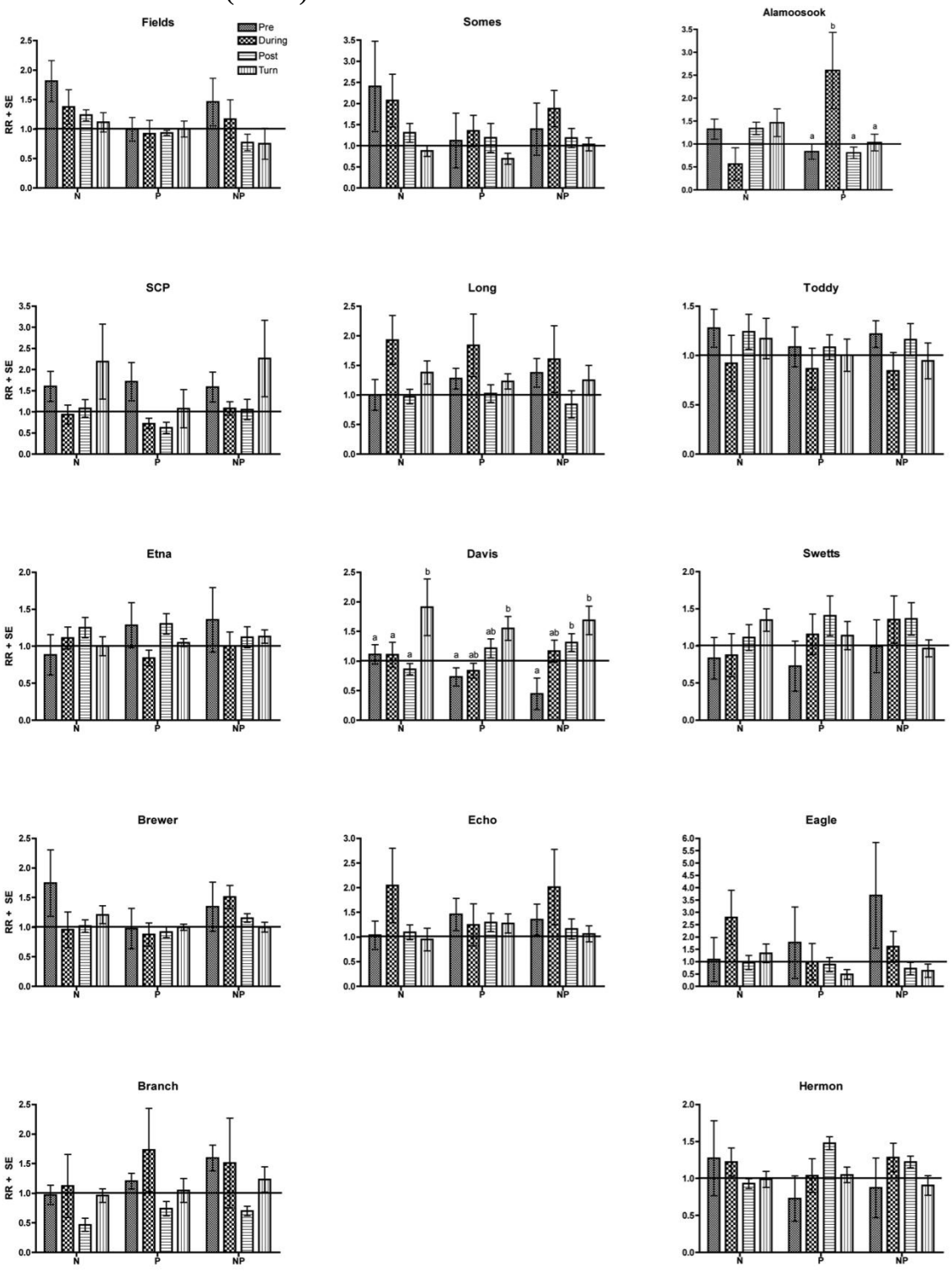


Figure A. 2. Individual stream ‘treatment by season’ results for RRs. Significant differences between seasons for each individual treatment (P<0.05) are marked with letters. Treatments with no letters indicate no differences in RR between any of the four seasons. Solid horizontal lines represent no change in treatment when compared with controls (RR=1).

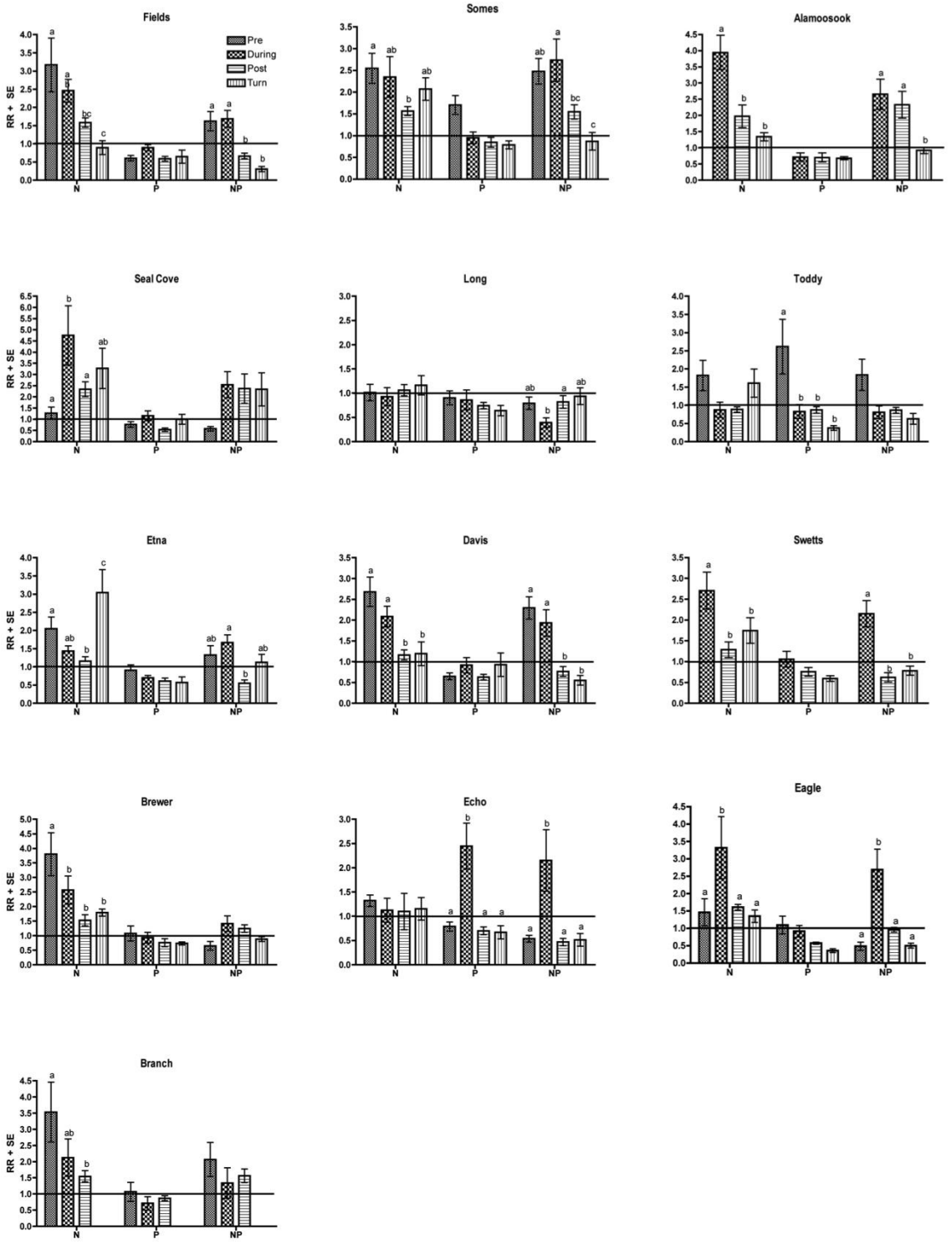
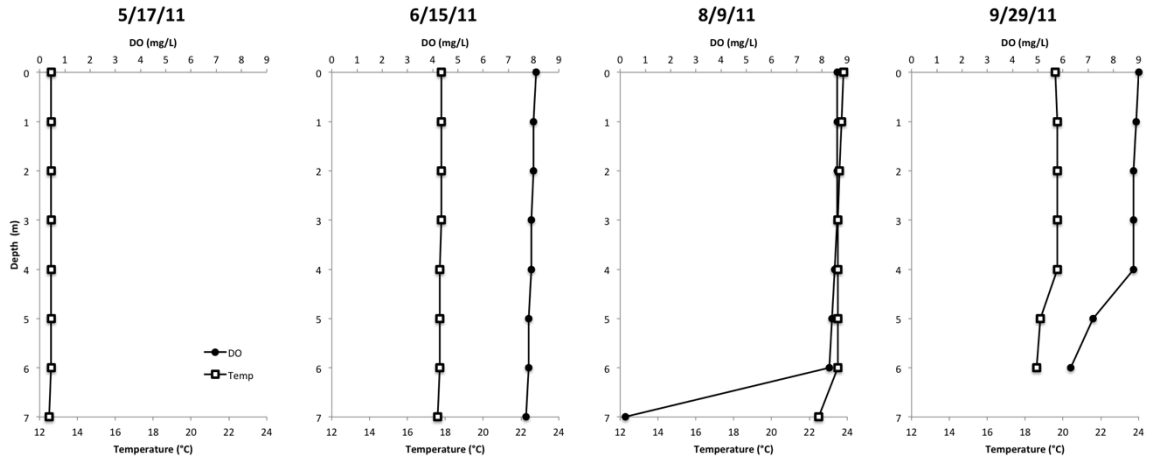


Table A. 3. Comparison of stream chl*a* and AFDM limiting nutrients. All limiting nutrients were determined based on NDS chl*a* and AFDM values with two-way ANOVA. $P<0.05$ unless designated with a ~ indicating it was marginally significant ($P=0.06$). No significant limitation is denoted ---, NP means co-limitation, N, 2P means primary N secondary P limitation, P, 2N means primary P secondary N limitation and # means there was no data for that site or season. Each limiting nutrient has been color coded to assist with visual differentiation of shifts in limitation.

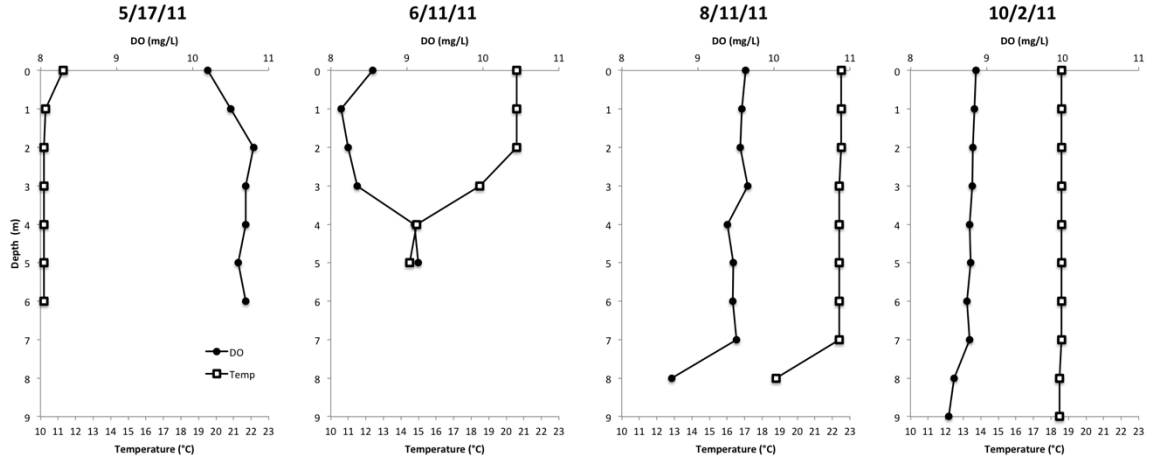
	Chl*a* Stream				AFDM Stream			
Stream	Pre	During	Post	Turn	Pre	During	Post	Turn
Alewife								
Fields	N	N	N	--	P, 2N	---	#	~N
Somes	NP	N	N	--	N	---	---	NP
Alamoosook	--	N	N	N	#	~NP	P	NP
SCP	--	N	N, 2P	N	NP	---	~P	N, 2P
Long	--	--	--	--	NP	NP	NP	N
Toddy	P, 2N	--	--	--	NP	---	---	P
Hermon	#	#	#	#	#	#	#	#
Non-Alewife								
Davis	N	N	--	--	NP	N	N	P
Swetts	--	N	--	N	#	P	---	N
Brewer	NP	N	N	N	NP	NP	#	---
Echo	N	P	--	--	NP	---	---	P
Eagle	P	N	N	--	NP	NP	N	---
Branch	N	N	N	---	NP	~N	P	#
Etna	N	N, 2P	--	N	NP	~NP	---	NP

Figure A. 3. Lake temperature/dissolved oxygen depth profiles. Graphs are shown for each of the four sampling dates.

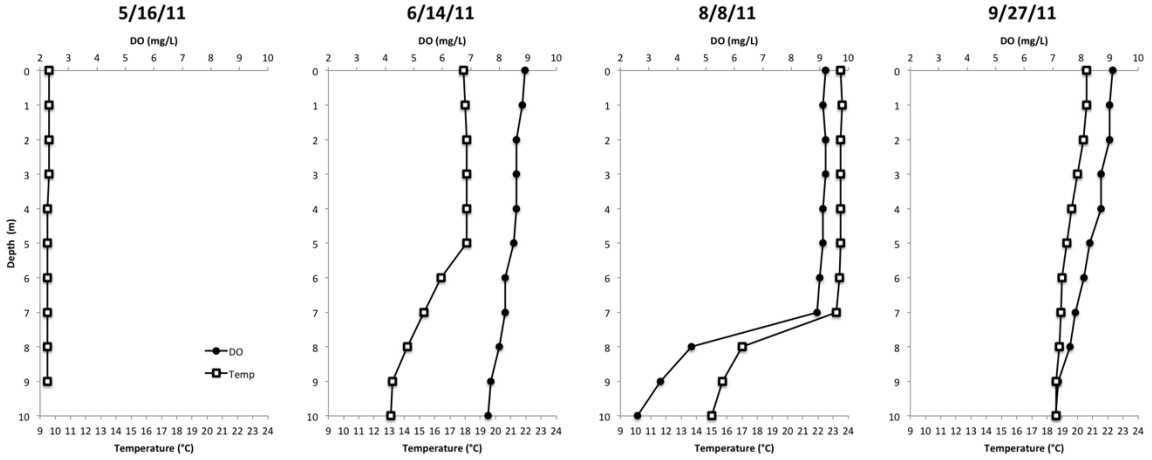
Alamoosook



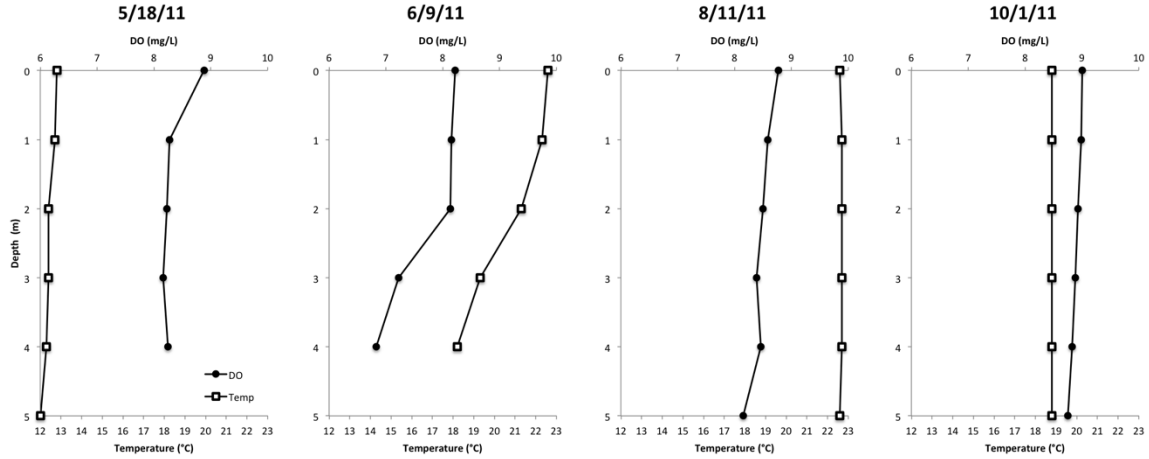
Branch



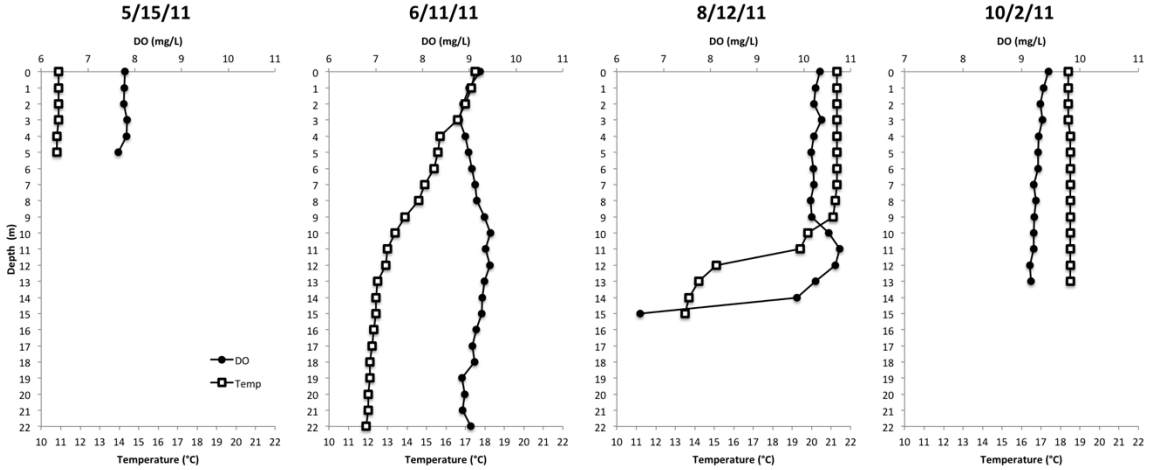
Brewer



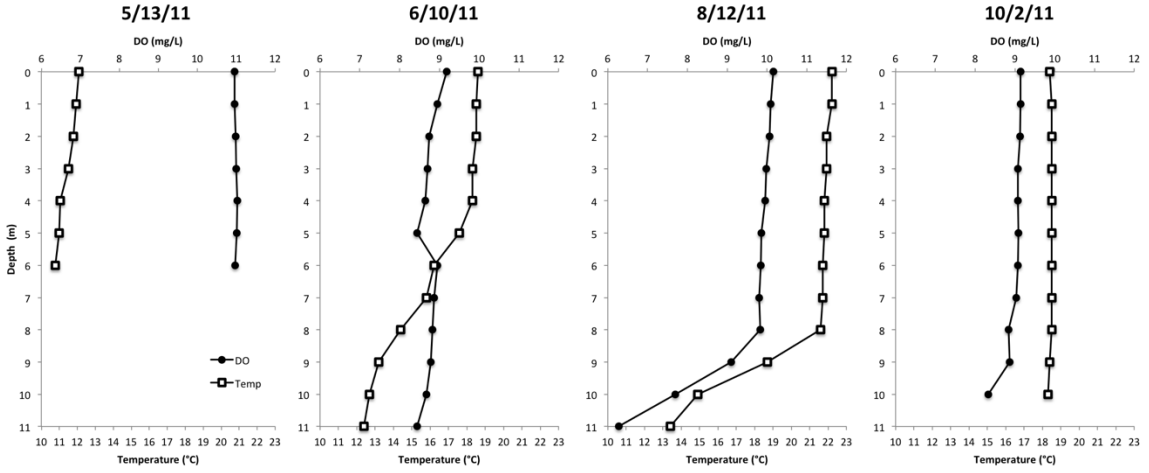
Davis



Eagle



Echo



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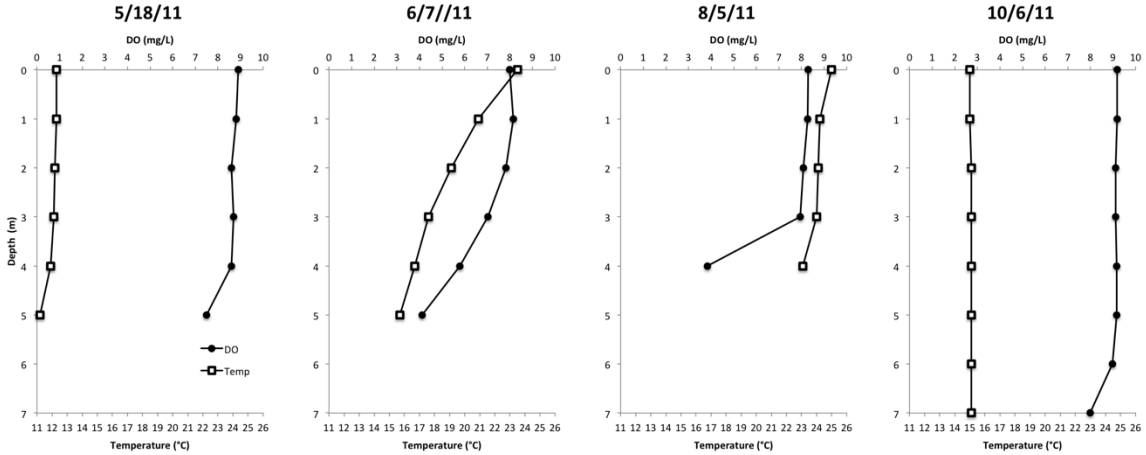

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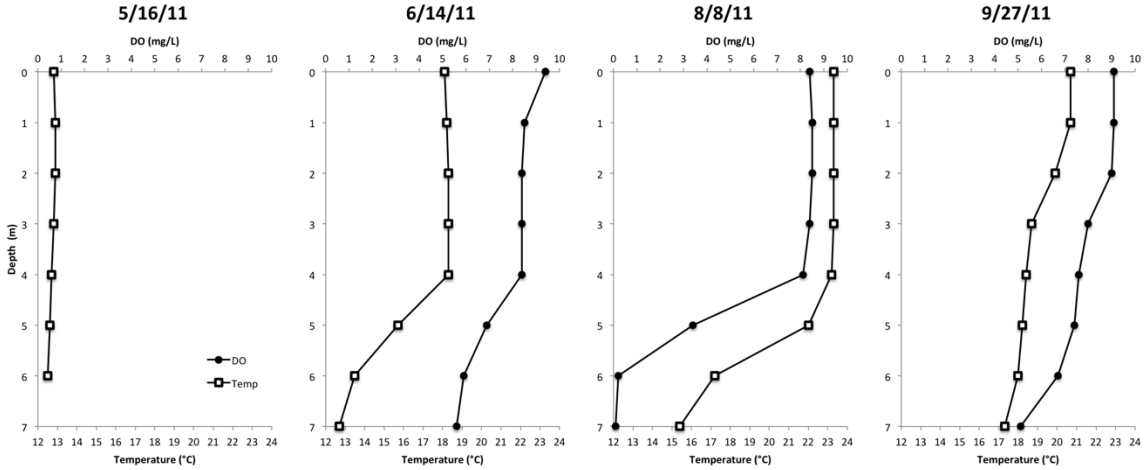
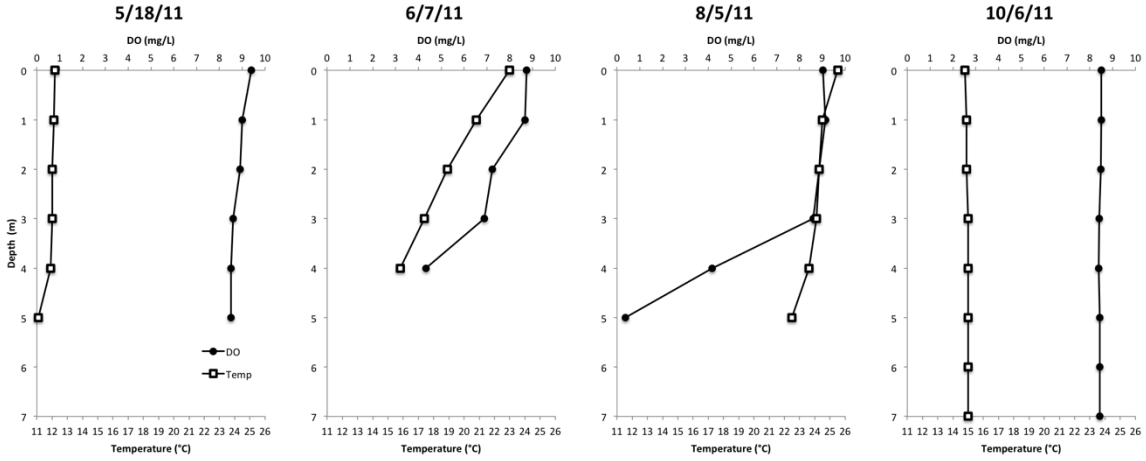
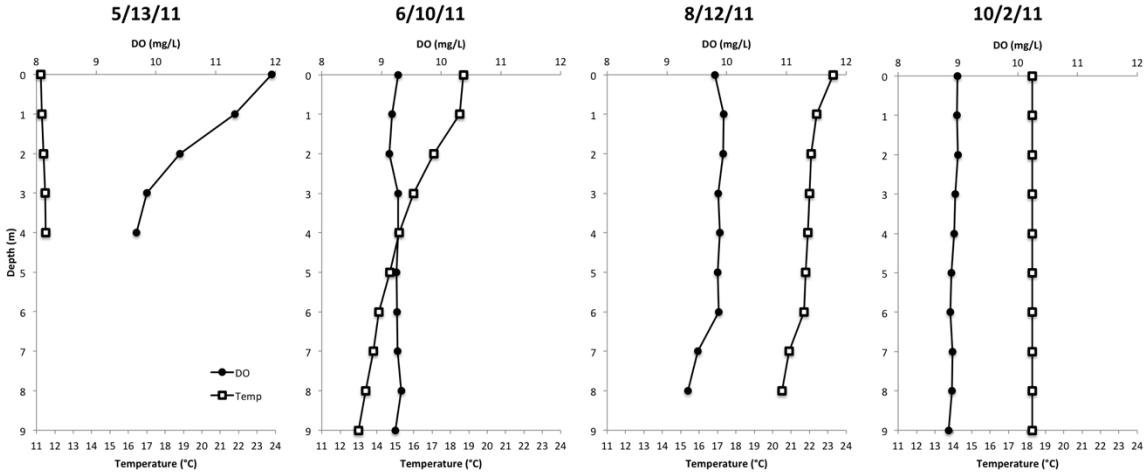


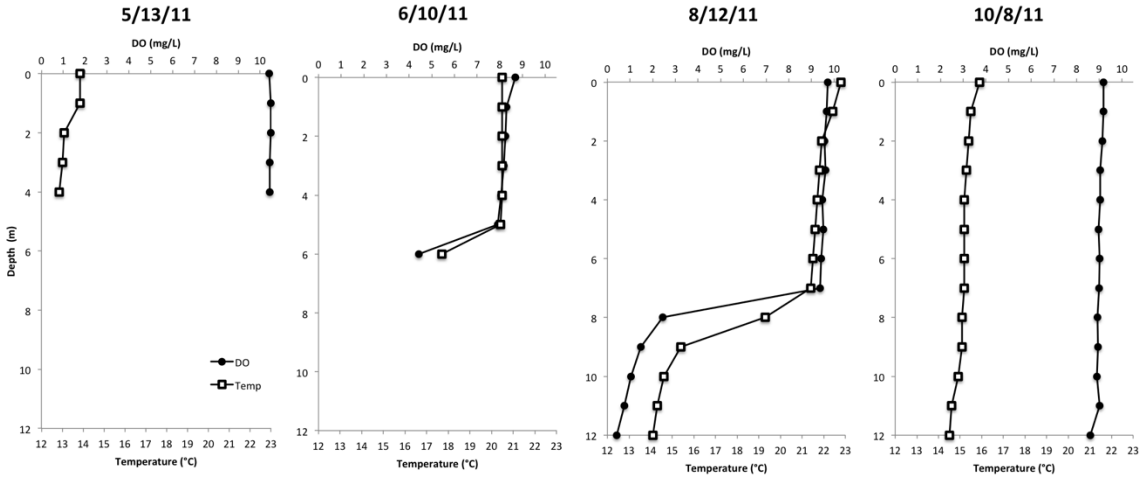
Hermon



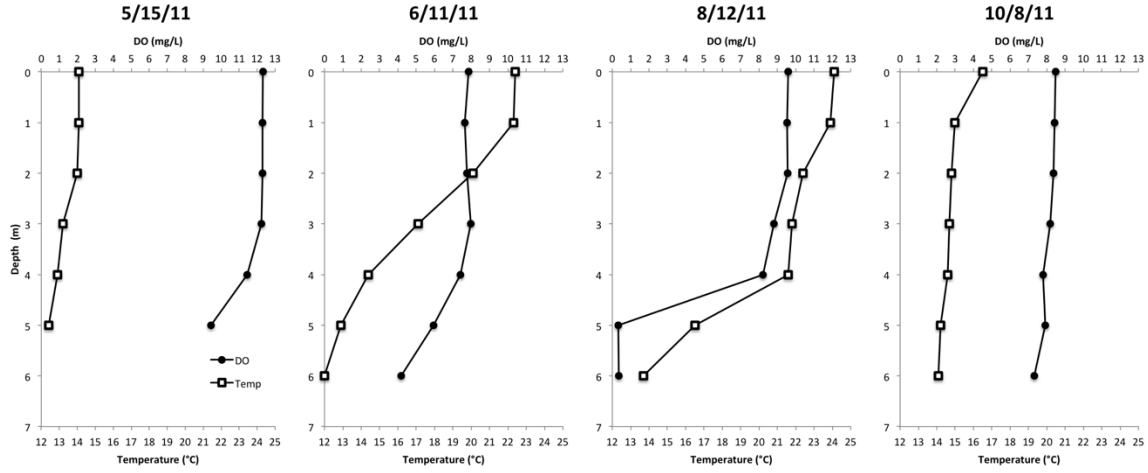
Long



Seal Cove



Somes



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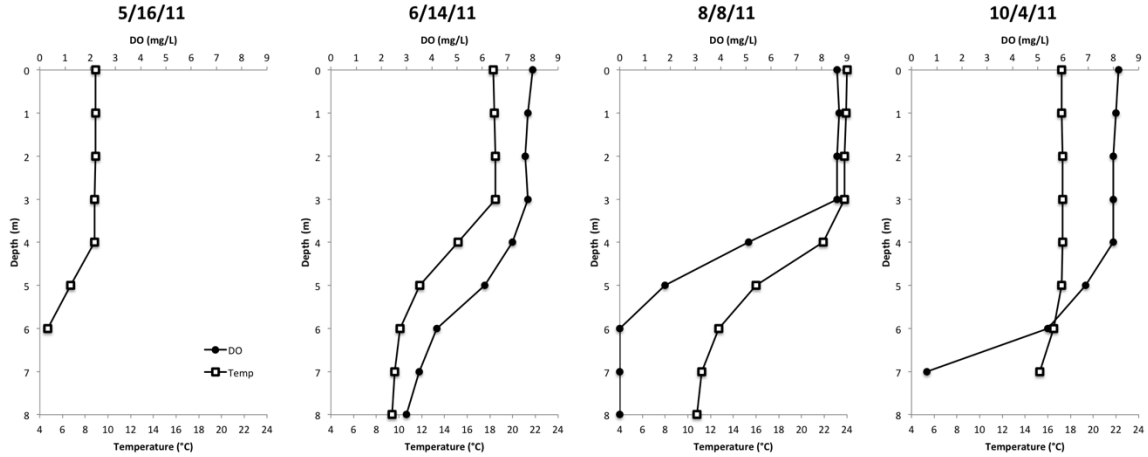
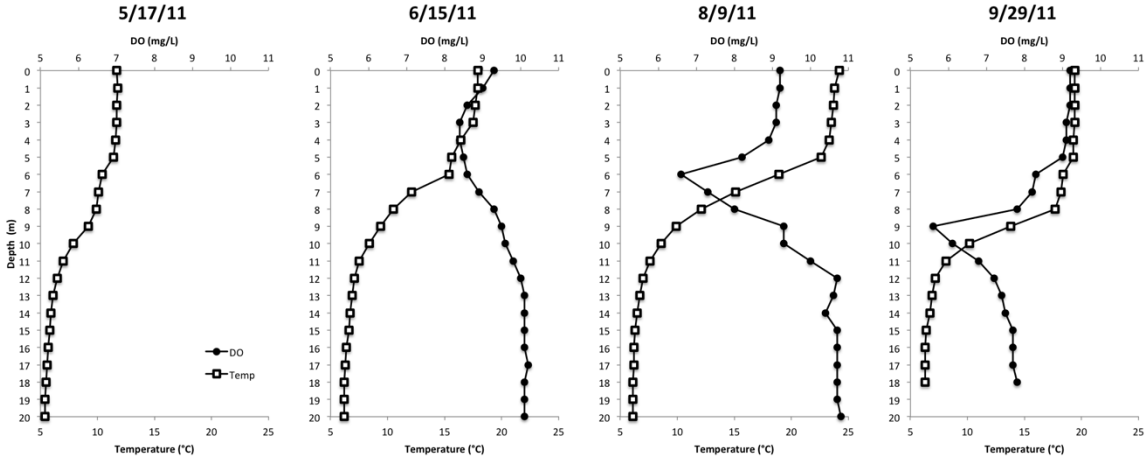


Toddy



BIOGRAPHY OF THE AUTHOR

Katie Norris was born in Cincinnati, Ohio on January 29, 1988 to Renée and Steve Norris. She has two older brothers, Rick and Drew. She was raised in Dent, Ohio along Taylor creek, and graduated from Oak Hills High School in 2006. She attended the University of Dayton and graduated in 2010 with a Bachelor's of Science degree in Interdisciplinary Environmental Science. She then entered the Ecology and Environmental Science graduate program at the University of Maine. Katie is a member of the Society for Freshwater Science (formerly the North American Benthological Society), and the Ecological Society of America. Upon graduation from the University of Maine, Katie plans to continue exploring the world, learning in many ways, and using her knowledge of water resources for the common good. She is a candidate for the Master of Science degree in Ecology and Environmental Science from The University of Maine in May, 2012.