Report to Maine DEP and Project SHARE

The Use of Clam Shells for Acid Mitigation in Maine Salmon and Brook Trout Streams.

Second Annual Report for Project SHARE, on the Dead Stream-Bowles Lake Stream Clam Shell Liming Experiment

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Contact: Mark Whiting, Biologist
Phone: (207) 356-5977

MAINE DEPARTMENT OF ENVIRONMENTAL PROTECTION
17 State House Station | Augusta, Maine 04330-0017
www.maine.gov/dep

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Executive Summary

Acid rain and intensive forestry have acidified soils in eastern coastal (Downeast) Maine and are among the factors contributing to a lack of Atlantic salmon recovery. Project SHARE is using clam shells as a calcium carbonate supplement to mitigate stream acidity and to improve freshwater salmon survival. In 2010, 2 metric tons of shells were placed in Dead Stream. In 2011, the treatment was expanded into the southern part of the watershed (Bowles Brook) and increased to 10 tons of shells for the entire watershed. Water chemistry improved by approximately 0.75 pH unit. Brook Trout, the most abundant fish present, have increased by almost three-fold, apparently due to a combination of better access above road crossings and to the improved water quality.

Bowles Lake Stream (Bowles Brook) with mahogany clam shells

I. Introduction

Project Background:

Project SHARE is a partnership between private landowners, local land trusts and conservation groups, with state and federal environmental and wildlife agencies involved in the restoration of Atlantic salmon in eastern Maine (see Project SHARE website http://salmonhabitat.org/home/). In November of 2009, Project SHARE was granted a Maine Pollutant Discharge Elimination System (MEPDES) permit #ME0002704, Maine Waste Discharge License (MDL) Application #W-009049-5Z-A-N **Project SHARE Final Experiment Permit, T37, T31, T30 MD**. This permit allowed clam shells to be placed in the Dead Stream- Bowles Lake Stream watershed as a 5-year experiment to mitigate for episodic acidification of these salmon streams. If successful, fish habitat will be improved, fish will be healthier, and improved survival will result in more fish and improved densities. The permit requires water quality monitoring and an annual report to Maine Department of Environmental Protection (DEP). The first field season was 2010, so this report is the second annual report and the first full year after the initial shell applications to Dead Stream at the 55-00-0 Road.

Project Scope:

Wild sea-run Atlantic salmon in Maine are in trouble. Both freshwater and marine survival are poor. Maine sea-run Atlantic salmon populations are maintained only with a vigorous hatchery stocking program. In order to make salmon populations self-sustaining, both freshwater and marine survival must be improved. In freshwater, there are water quality problems from a combination of related factors, including acid rain and more than two centuries of intensive logging. The effect on soils has been to deplete base cations from the most susceptible watersheds, resulting in chronic or episodic acidification of streams, low buffering capacity, low calcium concentrations, and high aluminum concentrations. The effects on fish are poor fish condition and low survival. Adding calcium carbonate to nursery steams is believed to be a possible remedy. The project plan is to use clam shells as a source of calcium carbonate to improve water quality. So far, *Mya arenaria*, the common "steamer clam" and *Arctica islandica*, the "mahogany clam" or "black quahog" have been used. The shells are a waste product from Maine's seafood industry and have been composted to minimize organic material that would decompose in the stream and could result in serious loss of dissolved oxygen. These shells have the additional benefit of having a large complex shape that would not cause embeddedness of fish habitat (i.e., the filling of interstitial spaces in stream gravel with fine sediments). Furthermore, the voids between the shells provide habitat for invertebrates and for young fish.

Dead Stream is scenic and typical of the eastern Maine brook trout and salmon streams. However, the mainstem is chronically acidic with typical summer baseflow pH between 5.5 and 5.8. The stream is also subject to acidic episodes with pH in the high 4's following storms. In 2009, Project SHARE improved fish passage in this watershed by removing old mostly undersized culverts and replacing them with engineered arch culverts. These culverts maintain a fish-friendly and natural-looking stream bottom under road crossings. Maine Department of Marine Resources (DMR) is also adding woody debris (logs and root wads) as an experiment in improving fish habitat in a study reach in the lower watershed. Salmon are stocked in this same general area below the 58-00-0 Rd (Figure 1), and occasionally at other sites when there are extra salmon fry. The clam shell liming project was designed to complement other fishery improvements in this Project SHARE work "concentration area."

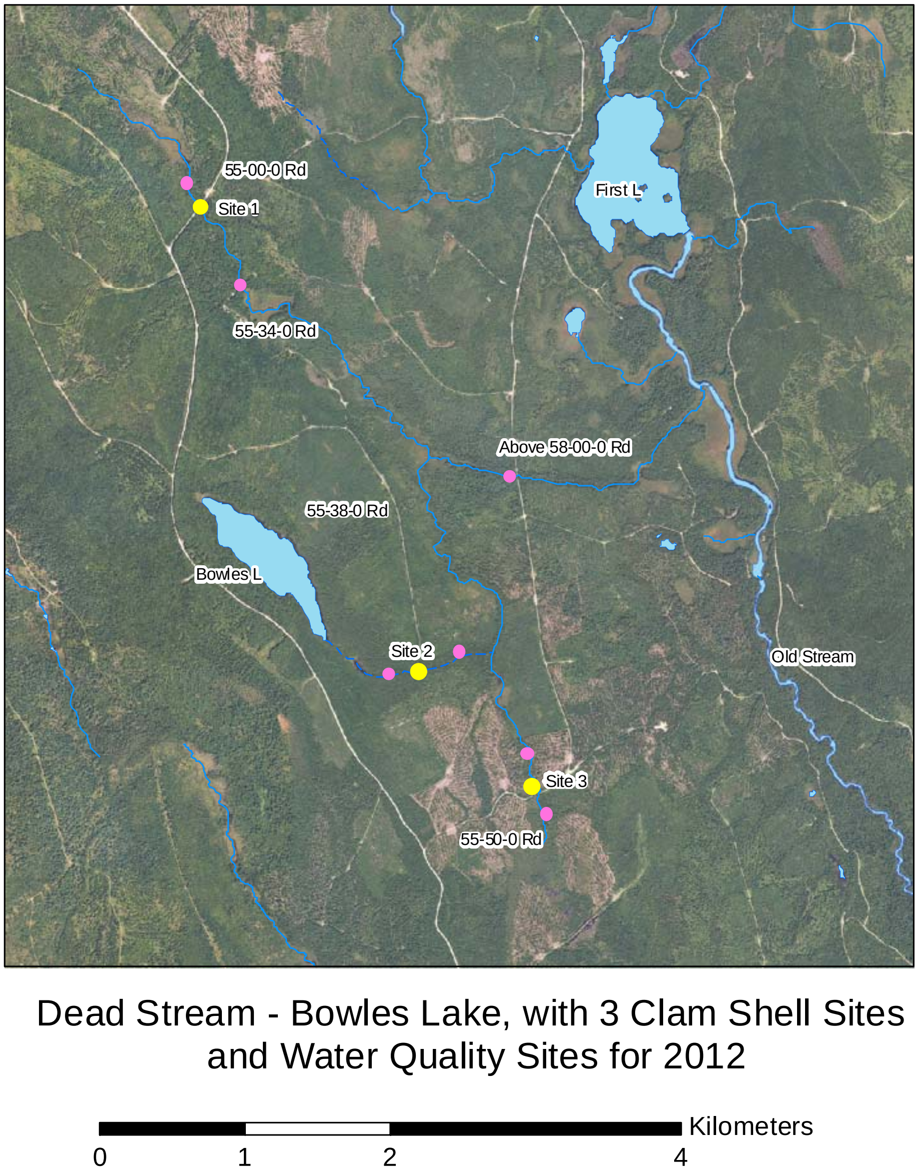

Figure 1. The Dead Stream-Bowles Lake Stream watershed is shaped like a "T" that is turned over on one side. The northern branch is the mainstem of Dead Stream. The southern watershed is the outlet of Bowles Lake that was called Bowles Lake Stream. An un-named tributary to Bowles Lake Stream is accessible from the 55-50-0 logging road. The combined branches of Dead and Bowles Lake Stream flow into Old Stream, one of the larger tributaries of the Machias River. Three clam shell application sites are marked in yellow. Seven monitoring sites are indicated in red and are located above and below

the shell application sites and one downstream monitoring site below the stream confluence on the 58-00-0 Rd.

The original acid mitigation Experimental Permit (dated November 2009) was modified to allow three shell application sites in the Dead Stream watershed for the 2011 field season. These sites are: (1.) the mainstem of Dead Stream between the 55-00-0 and 55-34-0 Roads, (2.) on Bowles Lake outlet (hereafter referred to as "Bowles Lake Stream") on the end of the 55-38-0 Rd, and (3.) on an unnamed tributary to Bowles Lake Stream on the 55-50-0 Rd (Figure 1). All three sites are located on perennial first order streams. Water quality monitoring occurred above and below the application sites and farther downstream at a monitoring site on the mainstem above the 58-00-0 Road. Authorization to expand the experiment to sites to other watersheds is conditional on the outcome of the initial experiment on Dead Stream-Bowles Lake Streams.

Project SHARE is permitted to put a total of 2 metric tons of clam shells in Dead Stream (Site 1) at the 55-00-0 and 55-34-0 Road access points, 2 metric tons at Bowles Lake Stream (Site 2), and 6 metric tons into the un-named tributary at the 55-50-0 Road crossing (Site 3). The target was about pH 7.0 based on comparisons with other streams in the Machias River watershed. For instance, streams with a circumneutral pH (like nearby Lanpher Brook) have enough buffering capacity to avoid episodic acidification (i.e., temporary pH declines below 6.0).

The Acidity Problem:

Large parts of Maine have both terrestrial and aquatic ecosystems that are strongly affected by acid rain. For instance, recent modeling results from Ecosystems Research, a contractor working for US EPA, USGS, the National Park Service and several state environmental agencies show that the northeastern United States in general is vulnerable to acid rain (Miller 2012). The acid rain Critical Load model was originally applied as a terrestrial model to show where forest growth is likely to be limited by soil acidification. The "Critical Load" is when the losses of base cations (calcium, sodium, potassium and magnesium) from forest soils (a "loss" function due to leaching and cation ion exchange) due to acid rain (the "loading" input variable) and forestry harvests (a second "loss" and export function) exceed the rate at which soils can replenish themselves due to bedrock weathering. This model was recently extended to show aquatic exceedances (Figure 2) (again the Critical Load is when the cation losses exceed the replenishment rate) (for more details refer to Miller 2012). According to the model, approximately 35% of Maine is currently experiencing terrestrial and/or aquatic exceedances. With severe exceedances (orange areas on the map), ecosystems would be expected to experience severe disruptions such as loss of species diversity and functional impairments. Some losses of species and changes in food webs would occur at lesser impacts (yellow and light green). Stream impairments vary from chronically acidified, to episodically acidified, to not acidified but experiencing losses of ANC and calcium.

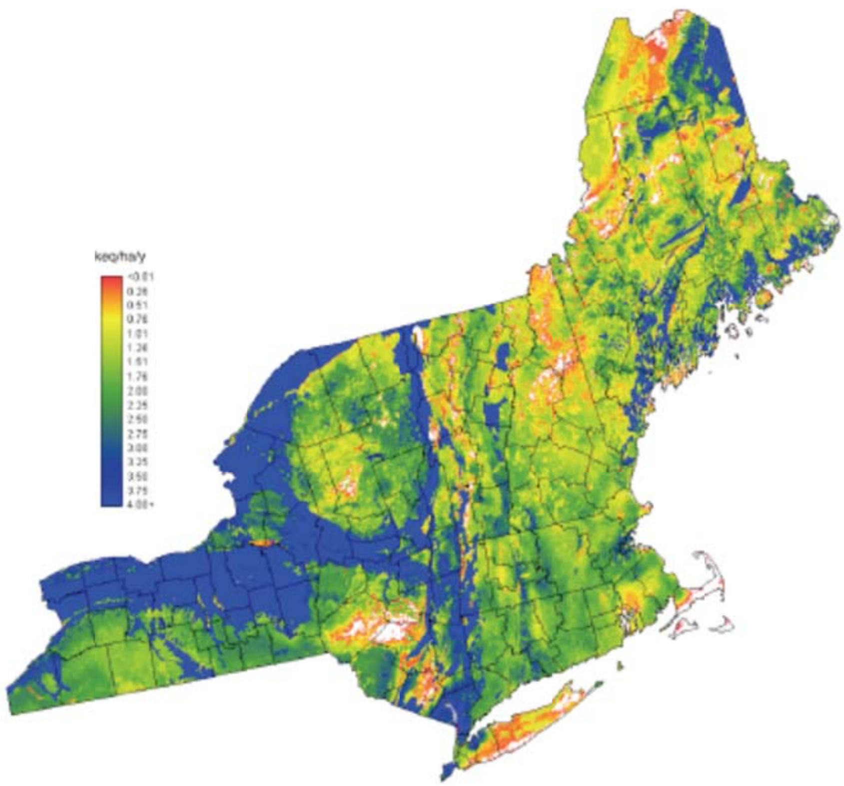

Figure 2. The results of the Critical Load modeling for the Northeastern US for aquatic ecosystems by Ecosystems Research Group (Miller 2012). Orange is where Critical Loads are strongly exceeded (i.e., where leaching of cations due to acid rain and export of cations due to forestry exceeds the local capacity to replace cations through weathering of bedrock). When the Critical Load is strongly exceeded, lakes and streams are expected to become chronically acidified. White is where soil base saturation is so low that the target pH cannot be reached (naturally acidic areas, and sandy soils or ledge). Yellow is the next most severe acidity change, where episodic acidification is expected and some species losses are expected. Light green is a moderate concern, i.e. the most vulnerable species of fish and zooplankton may be lost, and juvenile fish may experience sub-lethal stresses. Dark Green and Blue are of low risk.

Only the blue areas have enough bedrock weathering to completely neutralize acidic inputs (mainly limestone bedrock, marine clay and/or deep soils).

Acid rain is significant for the wide-ranging impacts it has on ecosystems. For instance, acid rain will (1) increases toxic ions such as H+ and aluminum and decreases nutrient cations like calcium and potassium, (2) mild acidification decreases species diversity by eliminating the most sensitive organisms, (3) severe acidification causes ecosystem level dysfunction as whole groups of organisms are eliminated and as biogeochemical cycles are disrupted (Jenkins et al 2005). Different parts of the ecosystem change in different ways. For instance in sensitive areas, acid rain alters soil fertility by cation depletion, accumulation of nitrogen and sulfur in soils and organic litter, and the mobilization of iron, aluminum and phosphorus (Driscoll et al 2003). Acidification changes the ecology of soils. Microbial activity, species diversity and growth are affected by soil pH and toxic metals (Francis 1986). Bacteria are more sensitive than fungi, except for the thiobacilli (which are tolerant of environmental extremes). Forest mycorrhizae are vulnerable (ESA 2011), resulting in reduced coupling between nutrient regeneration and nutrient retention in forest soils. Biogeochemical cycles are disrupted, especially nitrogen-fixation and nitrification (Francis 1986) and forests become exporters of nutrients. Acidification changes leaf litter and dissolved organic matter in soils, enriching it with nitrogen thereby making it more hydrophilic and less easily digested by microbes (McDowell et al 2004). Because soil acidification is slow, the microbial community has time to adapt. There is a shift to more tolerant species with variable results on microbial biomass and soil processes (Francis 1986).

Forest health can be affected. Acid rain leaches nutrient cations from the leaves of red spruce, making the leaves vulnerable to frost damage (Driscoll et al 2003). Mortality of sugar maples appears to be due to the weakening of the tree due to cation losses, and subsequent stresses such as insect damage and drought sensitivity (Driscoll et al 2003). There are some data that suggests that calcium losses in cation-poor soils can affect food chains resulting in the thinning of egg shells for song birds (Graveland et al 1994).

Surface waters are affected as acid rain leaches base cations, such as calcium, sodium and potassium, from soils (Lawrence 2002). As soils acidify, lakes and streams lose their primary source of alkalinity. Acidification of surface water occurs first as storm-related episodes, and then as it progresses acidification can become chronic. Aluminum is converted into forms toxic to fish and other aquatic organisms when the pH declines below pH 6.0. In addition to terrestrial and freshwater problems, there are links between soil acidification and the eutrophication and mercury contamination of estuaries and the coastal ocean (Driscoll et al 2003).

In freshwater streams and lakes, both plants and animals are affected. In southwestern Sweden, acidified lakes became overgrown with *Sphagnum* moss and benthic algal mats. Rooted plants are also affected, including species losses and shifts in dominance within the plant community (Grahn 1986). Phytoplankton abundance in acidified lakes is often reduced, not because of toxicity (tolerant species will replace less tolerant ones) but due to the binding of phosphorus with aluminum into forms that are not biologically available (Hendrey 1982). In contrast with soil microbes, fungi in streams are impaired in acidified streams. In a literature review, Hendrey (1982) found that fungal biomass on leaf packs in acidic streams was only 3-9% that of control streams, and that acidified lakes accumulated abnormal amounts of detritus.

Benthic animals in streams are also strongly influenced by pH and aluminum. Many macroinvertebrates are used as indicator taxa for acidification (Økland & Økland 1986, Moe et al 2010). Amphipods, some crayfish, crustaceans, mollusks and most mayflies are among the most sensitive taxa (Økland & Økland 1986, Simpson et al 1983). For instance the Percent Mayfly Richness (PMR) index is incorporated into the Acid Biological Assessment Profile (acidBAP) in Adirondack and Catskill Mountain stream assessments (Lampman et al 2008, Burns et al 2008). Also the Number of Ephemeroptera (mayflies) Families and the Proportion Sensitive Ephermeroptera are used in Europe as acidification indices (Moe et al 2010). In terms of macroinvertebrate guilds, streams that are acidified have generally lost their grazer community (Sutcliffe & Carrick 1973). This ecological function is replaced to some extent by the trophic generalists (the so-called “scrapers” which feed on detritus, microbial biofilms and algae) (Ledger et al 2000). The widespread decline of calcium in soils and surface waters of the Canadian Shield due to acid rain and logging, have resulted in dramatic shifts in zooplankton communities in lakes, including the loss of the water flea *Daphnia* (Jeziorski & Yan 2006). In fact, variations in aquatic invertebrate assemblages with acid-base status are among the most repeatable patterns in stream ecology (Sutcliff & Carrick 1973).

A wide range of tolerances are found among fish species (Simonin et al 1993, Reckhow et al, 1987). Fish fry and young-of-the-year are often the most vulnerable life stage (Baker & Christensen 1991) and in salmon the smolt stage (the young fish adapting physiologically for life at sea) is the most sensitive (Staurnes et al 1993). Reproductive failures lead to the losses of year classes, and sometimes the only remaining fish are aging adults (Brezonik et al 1993). In southern Norway, 18 rivers in the southern part of the country (thereby receiving the worst acid rain from central Europe) have lost their Atlantic salmon (Sandoy & Langaker 2001). The earliest salmon losses date back to the 1920’s. An aggressive liming program has allowed these commercial fisheries to be restored as the European community addresses air pollution controls. In the Netherlands, the number of lakes with amphibians and amphibian species diversity has declined as acidification progressed (Leuven et al 1986). In other words, acid rain has ecosystem-level effects at all taxonomic levels from Kingdoms to individual species.

Because the freshwater bicarbonate buffering system works well from pH 6.5 to 8.3 (or alkalinity - acid neutralizing capacity (ANC) above 250 ueq/L), acid rain is thought to mainly affect surface waters that are more dilute or "softer." The effectiveness of the bicarbonate buffer deteriorates quickly below pH 6.5 (note the location of zero ANC in Figure 3 and the steep slope of the line). The lower part of the pH – Alkalinity curve is difficult for most aquatic organisms. Since alkalinity comes primarily from the soil, strong rains will dilute alkalinity and cause pH to fall with each passing storm. The pH swings during and after each storm event can be 1-2 pH units (up to 100 times the starting acidity level). The pH falls quickly, usually within a matter of hours and then takes several days to recover (approximately a week in all, even for small streams). As surface waters lose buffering capacity, they can become chronically acidic. The problem is analogous to an estuary, with freshwater on one end and seawater on the other. Many organisms are adapted for one or the other, but adaptations to the extremes found in between are rare. Many species are transient, invading on one side or the other with the ebb and flow of tides. The transients leave when the flow shifts the wrong way for their adaptation.

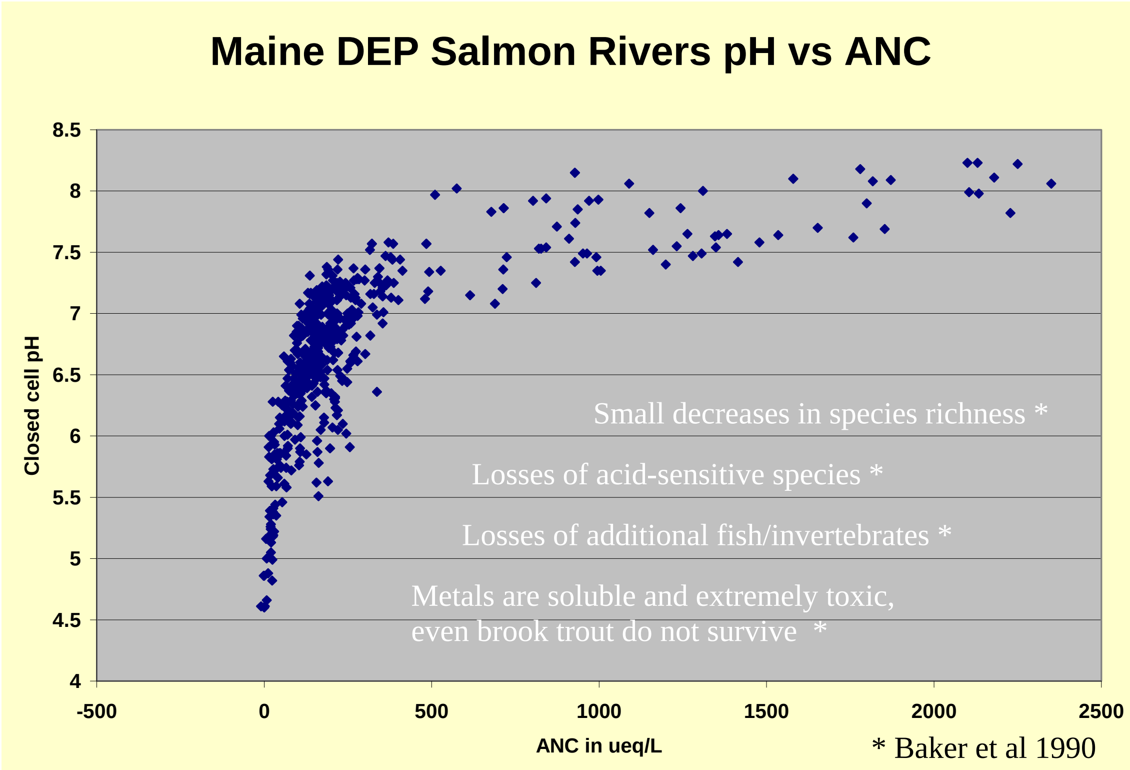

Figure 3. Stream pH and Alkalinity (ANC) are plotted for more than 600 samples from 50 streams and rivers sampled in the Maine DEP Salmon Rivers Program. Critical pH thresholds have been taken from studies in the Adirondacks (Baker et al 1990) and are provided for reference. Some losses of species diversity begin when normal summer baseflows fall between pH 6.0 and 6.5 (rainbow smelt, alewife, slimy sculpin and many minnow species are in this category). The pH swings during high flows become increasingly severe below this value (note on the chart how small changes in ANC result in large pH changes). The bicarbonate buffering system in this range is becoming increasingly less effective and depleted (note 0 ANC on the chart). Additional species losses are expected when baseflow pH falls between pH 5.5 and 6.0 as acid-sensitive species can no longer reproduce when juvenile fish (and Atlantic salmon smolts) are killed during acidic episodes. Even acid tolerant species may not survive when baseflow pH falls between 5.5 and 5.0 (again eggs or juveniles may be the most vulnerable). Below pH 5.0 even adult brook trout cannot survive due to toxic aluminum and other metals.

Streams are more vulnerable than lakes to acidification since they are more directly influenced by runoff and have less water storage (Lampman et al 2008). Lakes may have water residence times that range from months to years, thereby integrating the effects of many individual rainstorms. Streams have residence times of a few hours and rivers a few days (except for in-stream lakes and impoundments) and are strongly influenced by individual storms. Furthermore, streams typically become acidic beginning in their headwaters and proceeding downstream (Lawrence 2002). For instance, in the Catskill Mountains of New York, the stream length that is affected by acidification changes from 15% at baseflow to 82% during high flow episodes (Lawrence 2002).

Streams and lakes that are too acidic to support native communities are typically treated with limestone (calcium carbonate sand or powder). Such liming projects are typically successful (see review articles in Haines & Johnson 1981, Brockson & Olem 1992). In West Virginia, sand-sized limestone was dumped directly on the stream bottom to treat acidified streams that were virtually fishless except for a few stray brook trout (Clayton et al 1998). The initial condition ranged from extremely acidic to moderately so (four streams with pH less than 5 and one stream with pH 5.7, zero ANC, calcium ranging 1.5 -2.8 mg/L, and high Alx 190-330 ug/L). The calcium carbonate dose was calculated the same way we did (we used their model) and ranged from 2X to 6X the calculated dose (the authors recommend using double doses for first-time projects, single doses are fine for maintenance liming). Fish populations rebounded in 3 years (from a biomass low of 0.1 kg/ha to a high of 38 kg/ha) and included eight fish species. The final pH ranged 5.5 -6.1, calcium ranged from 3 -9.3 mg/L, and Alx was greatly reduced (but still very high 60-130 ug/L, note that high calcium protects fish from the toxicity effect of aluminum, Brown 1983). Clayton et al (1998) report that dividing the dose between three different treatment sites was no better than a full dose at one site, and that the 6X dose offered no additional benefit compared to the 2X dose. The rate of dissolution of the limestone sand depended on particle size, distribution within the stream (riffle or pool), contact time, stream flow, gradient, and stream acidity. One drawback on using limestone sand is that it fills in habitat between gravel and stone cobble on the stream bottom (causing "embeddedness" of the substrate) which results in lower macroinvertebrate and fish densities within the treatment area (Clayton & Menendez 1996, Keener & Sharpe 2005).

So what makes a liming project successful? In Norway, the authors of a study of the invertebrates and fishes of 1095 lakes and 30 river watersheds decided that Atlantic salmon and brown trout were among their most vulnerable species. The authors believe that Atlantic salmon are possibly the best indicator of the acidity status of rivers, and brown trout are the best indicator of the acidity status of lakes (Lien et al 1996). Maine has the same problem, salmon are unable to sustain themselves under current conditions. If Maine streams are truly impaired from acidic inputs, then the addition of a base should fix it. In contrast to simple nutrient additions, which might increase the number of individuals (but perhaps result in fewer species), recovery from acid/metal toxicity should result in ecosystem level improvements as species that have been locally eliminated make a recovery. Algal blooms should disappear, and be replaced with a more balanced and diverse plant community. Acid sensitive plant and animal species should return. Natural reproduction of fish species that used to be common (like Atlantic salmon, minnows and other small fish) should resume. In Norway, fishery managers lime rivers and streams and salmon recover mostly on their own (although there are some private hatcheries and stocking). In Maine, fishery managers stock fish and are improving habitat, but until now have not acted to modify the water quality. If stocking alone is not working, then more must be done. An ecosystem level improvement in diversity (and especially salmon recovery) should demonstrate the need for a continued liming program.

II. Methods:

In the summer of 2010, two tons of shells were distributed on the stream bottom of Dead Stream at Site 1 at the 55-00-0 Rd. Stream pH increased 0.5 unit during baseflow conditions near the application site. It was not clear at the time if the farthest downstream site was also affected (Whiting 2010). For the 2011 field season, the experiment was expanded so that a total of 10 tons was distributed among the 3 sites (Figure 1). The shell application rates were calculated using the methods of Clayton et al. (1998). This method uses watershed size and the current summer baseflow average pH as input variables. The model was based on the authors' experience of restoring trout streams in West Virginia using sand-sized limestone. On the basis of a pilot study using clam shells (unpublished) this model provides a conservative dose estimate for Maine streams. A total of 10 tons of shell in this watershed comes close to the calculated dose of 12 tons for the whole watershed above the 58-00-0 Rd (1,282 hectares).

For the 2011 field season, shells were purchased from a compost facility in Addison, Maine. Both steamer clam and mahogany clam shells came with the mix. These shells were estimated to be about 95% mahogany clams, some steamers, and rarely lobster or blue mussel shells. The mahogany shells are a little larger, and are much thicker, heavier and stronger than the steamers. Experience with them indicates that mahoganies last about twice as long in flowing water as the steamer clams do (about 1 year as opposed to 6 months, Scott Craig, US Fish & Wildlife Service, unpublished data). The composting process left a small amount of black organic material associated with the shells. Because of this organic matter, recently composted shells should only be applied during normal to high stream flows. However, due to procurement problems, the shells had to be applied during normal summer baseflows (and dissolved oxygen was monitored daily till the next storm). The shells were delivered stream-side by dump trucks, and were then carried upstream or downstream from access points in 5-gallon buckets. Shells were distributed approximately 100 m upstream and 100 m downstream from Site 1 at the 55-00-0 Rd, and 100 m upstream of the 55-34-0 Rd (considered the downstream end of the Site 1 treatment). At Site 3, alder thickets prevented shells from being distributed farther than 60 total meters on the 55-50-0 Rd site. In this case, most of the shells here were scattered on the bottom of an old beaver pond located immediately upstream of the road. At Site 2, on Bowles Lake Stream, shells were scattered approximately 150 m downstream from the end of the 55-38-0 Rd.

Shells were scattered loosely on the stream bottom or were bagged in 40 lb "onion bags." Calculations show that one ton of mahogany clams is equivalent to about 110 5-gallon buckets. One half of the total dose was shoveled into plastic mesh onion bags for both Sites 1 and 2. The bags allow the dose to be adjusted by adding or removing bags. The bags were donated by Moosebec Mussels of Jonesport, Maine. All of the shells in the fishless tributary at Site 3 were distributed loosely on the stream bottom. This stream has an average summer baseflow pH of 4.5, so there was no concern about over-dosing this system. Labor was provided by summer interns from US Fish & Wildlife Service, the Downeast Salmon Federation, and Professor Sherrie Sprangers' salmon biology class at the University of Maine at Machias.

The monitoring plan includes both water quality and biological assessments (Table 1). Water quality was measured in different ways. Data sondes (YSI model 600 XLM) were used primarily to measure water temperature, specific conductance and pH. The sondes were deployed in the spring and were programmed to record hourly during the field season. The sondes were recovered in November 3, 2011 before the logging roads closed in the fall. The sondes were recalibrated monthly, so field measurements "grabs" of conductivity (Oakton EC tester), pH (YSI 100 EcoSense meter) and alkalinity (LaMotte ANC titration) were collected at the same time. Water samples were collected quarterly (June, August, and October) for lab analysis, primarily for pH, calcium and aluminum species. Due to the occurrence of organic matter among the shells and worries about possible low oxygen, dissolved oxygen levels were monitored using a Hach HQ30d optical DO meter.

Table 1. Summary of monitoring plan for SHARE clam shell experiment. Maine Department of Environmental Protection, US Fisheries & Wildlife Service, and Maine Department of Marine Resources provided data.

Measurements	Method	Where	Analysis	When	Who
Water chem (pH)	Sonde	All Sites	before/after up/down stream	hourly, May-Nov	USFWS/DEP
Water chem (pH Ca Al)	Grab	All Sites	before/after up/down stream	quarterly	DEP
Water chem (field pH ANC)	Field meter Field titration	All Sites	before/after up/down stream	monthly	DEP
Algae	Grab	All Sites	before/after	yearly	DEP
Macroinvertebrates	Izaac Walton	All Sites	before/after	yearly	DEP
Fish abundance	E-fishing	Dead	before/after up/down stream	yearly	USFWS/DMR
Fish abundance	E-fishing	Crooked	before/after up/down stream	yearly	USFWS
Fish abundance	E-fishing	Honeymoon	before/after up/down stream	yearly	USFWS

The lab analysis included major cations (calcium, sodium, potassium, and magnesium) and aluminum species (total aluminum, dissolved aluminum, organic aluminum, and ionic (“exchangeable” or “labile” aluminum). The exchangeable Al (Alx) is the toxic form that is associated with acidification problems and causes damage to fish gills. At values below pH 6.0, any Alx value above 20 ug/L (parts per billion or ppb) can be harmful for young salmon if the exposure is long enough (McCormick and Monette 2007). For instance, it was found that Alx values of 39 ug/L and exposures that lasted for at least 6 days caused damage to fish gills and body salt balance. Values of 120 ug/L Alx can be lethal to salmon pre-smolts after 6 days of exposure in freshwater, or can result in failure to survive a transition to seawater (McCormick & Monette 2007). The presence of Alx in toxic amounts is an unambiguous indicator of anthropogenic acidification (ESA 2011). Or to put it another way, “measureable amounts” of Alx (in this case, given the uncertainties of measuring metals in parts per billion, “greater than 1.5 umol/L” or about 40 ug/L) “do not occur in the absence of acid deposition in streams that are otherwise undisturbed” (Lawrence et al, 2007).

Macroinvertebrate communities were evaluated using plastic mesh “rock bags,” also known as “riffle bags.” The deployment and retrieval methods were adapted from

Davies & Tsomides (2002). The macroinvertebrates were identified in the field using Izaac Walton League Save-Our-Streams (SOS) picture keys and data sheets. This rapid assessment technique is an adaptation of the more elaborate EPA rapid assessment procedure (EPA, 1997). Sorted and counted animals were identified and were returned to the stream. Given the importance of the macroinvertebrate response as a gauge of success, future macroinvertebrate assessments will be professionally contracted out.

Fish communities were examined with single pass e-fishing techniques. At the 55-00-0 Road the e-fishing study reach was 100 m above and 100 m below the road. Individuals were identified to species, and body length and weight were recorded. Fulton's K was used to combine body length and weight measurements into a single index of body condition (Fulton, 1902; Barnham & Baxter 1998). The larger or heavier the fish, the larger the value of K.

Algal communities were investigated using grab samples. Algae were scraped from rocks with a knife or bushed off with a tooth brush. Samples were observed by microscope in fresh condition (mostly at 500X) and diatom slides were made using an acid digestion (Patrick & Reimer 1966). Diatoms were observed under oil immersion at 1,250X magnification. Organisms were identified to genus in fresh material and to species in diatom mounts.

Weather is important in interpretation of water chemistry results because pH and dissolved salts are so variable with respect to stream discharge. Wet weather typically dilutes base cations and reduces buffering capacity, while increasing organic acids from contact with forest soils (Kahl et al, 1992). This drives pH down. Total aluminum increases during high flows as mineral particles are entrained by high water velocity and turbulence. Some of the total aluminum is converted into the toxic ionic dissolved aluminum, or "exchangeable Al," by the more acidic conditions. Rain and flow data were not measured by the project, but flow data are available from the USGS stream gauges. The closest one to Dead Stream is at Old Stream at State Route 9 (Figure 4). The field season started with a very wet May. June had normal flows while late July became hot and dry. After August 2, the weather was again very wet through the end of the year. Hurricane Irene struck the Maine coast on August 28 and delivered about two inches of rain in Washington County. Irene was one of several strong storms this year.

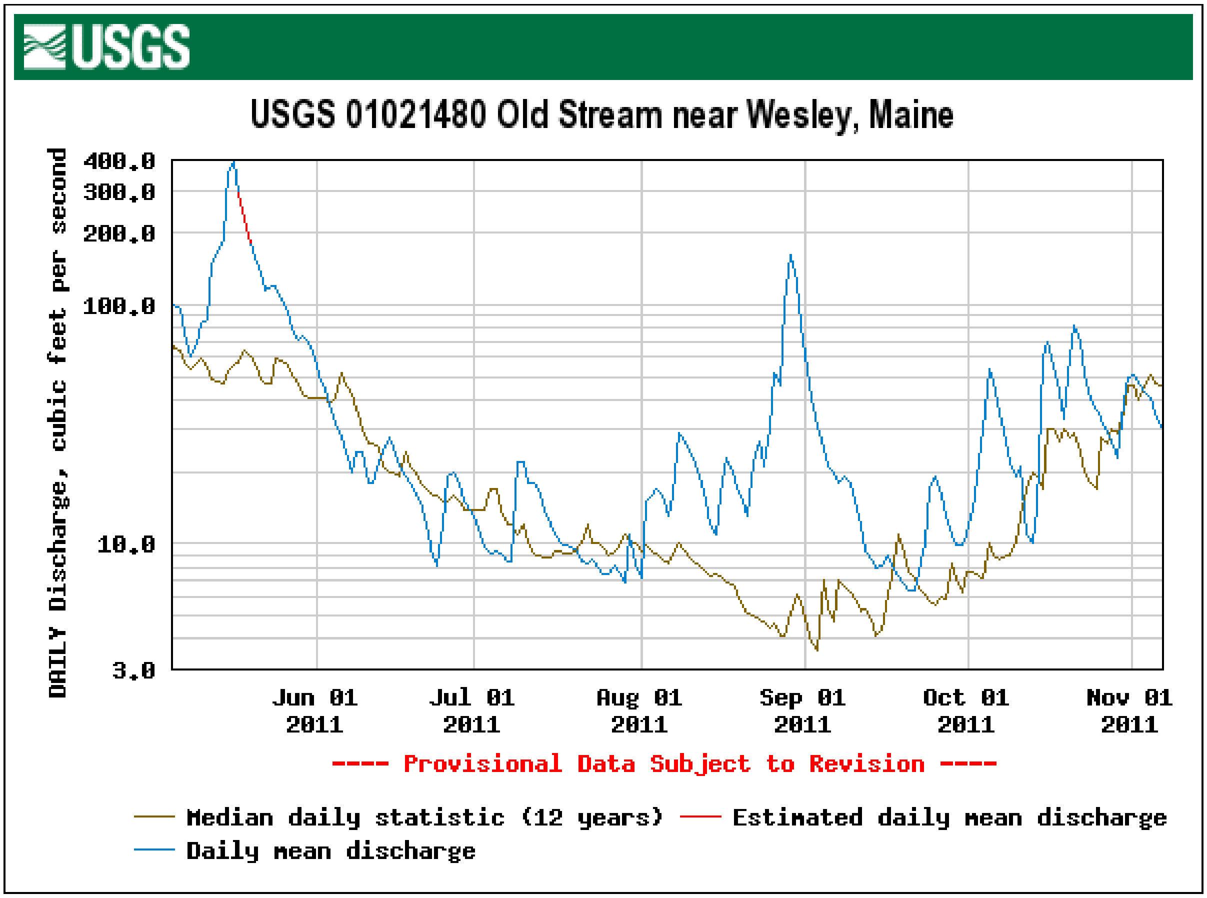

Figure 4. Stream flow data from the USGS gauge at Old Stream on State Route 9. Measured stream flow is in blue and the 12-year average flow is given in brown. Early summer was normal to dry, while May and late summer and fall were very wet.

III. Results & Discussion:

Stream pH responded immediately to shell additions and was recorded hourly by the data sondes. At Site 1, 2 tons of shells were added in the summer of 2010. In early May of 2011, a visual inspection of the site showed that most of last year's shells in riffle areas had completely dissolved. Some shells remained in pools and especially in the mesh bags. Water circulation through the mesh bags is poor because of the size of the bags and because the shells pack more densely as the shells dissolve and fragment. Figure 5 illustrates that the remaining shells from last year were adequate to keep stream pH about 0.5 pH unit above the upstream values. Another 2 tons of shells were added on July 7, 2011 and the pH increased another 0.25 units. Heavy rain drove pH back down again on July 9. Even during high flows, the pH remained above 6.0 at the downstream site (in red). Shells were added in the southern part of the watershed in the un-named tributary (Site 3) also on July 7. The final shell addition was at Bowles Lake Stream (Site 2) on July 20. The three shell application sites have a combined effect on Dead Stream below the confluence of the two subwatersheds at the 58-00-0 Rd. During baseflow conditions, the effect on the lower part of Dead Stream (light blue) represents an increase of about 0.25 pH unit.

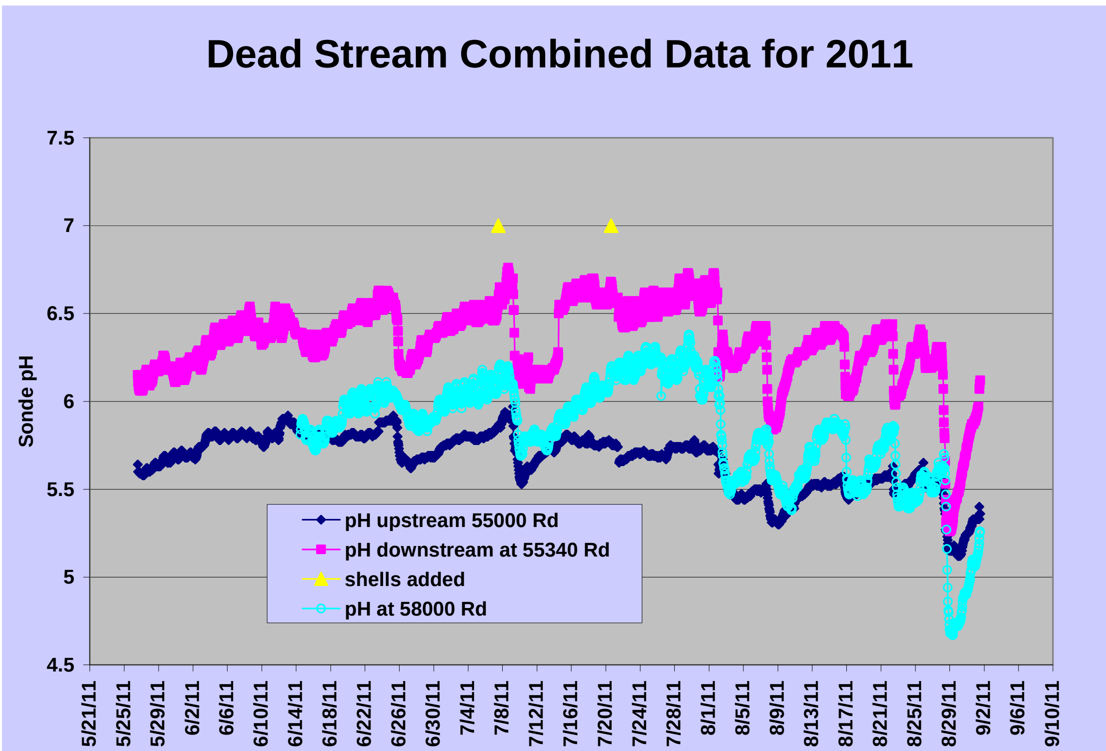

Figure 5. The sonde pH record for Dead Stream at the 55-00-0 Road upstream of the shells is superimposed on the record for the downstream site at the 55-34-0 Road. The upstream and downstream pH records are already 0.5 pH unit apart due to last year's shell applications. This figure includes the lower watershed monitoring site at the 58-00-0 Road for comparison with the upstream records. Shell addition dates are marked with yellow dots. Two metric tons of shells were added to Dead Stream (Site 1) on July 7, 2011. Six metric tons were added the same day to the southern watershed in the un-named tributary at Site 3 (sonde record not shown here). Two metric tons of shells were added to Bowles Lake Stream (Site 2) on July 20, 2011 (also not shown here). The combined effects of the three shell additions have a small effect on baseflow pH on the 58-00-0 Rd (i.e., the distance between the dark

blue and light blue lines is larger from July 20 through August 2 compared to earlier baseflow conditions). A series of August rain storms make pH comparisons difficult in the latter record (rain causes the dips in pH, and since the different parts of the watershed respond differently the reference point is lost).

The natural pH for Dead Stream at the 55-00-0 Road (upstream record) ranged from 5.6-5.8 during summer baseflow, and fell below pH 5.5 during high flows. This is a problematic range for Atlantic salmon. In Norway, salmon began to recover in acidified rivers as liming programs increased summer baseflow pH from 5.2 to 5.7 (Kroglund et al 2001a). After 30 years with no natural reproduction in these rivers, salmon fry and parr were noticed for the first time. The liming program was subsequently modified so that liming targets were pH 6.2 most of the year, but was increased to pH 6.4 during the spring salmon smolt run. Experimental evidence supports the liming program assumptions that a pH of 6.4 provides better protection from aluminum toxicity than pH 6.2 (Kroglund et al 2001b).

Evaluating Site 3 alone (Figure 6), the addition of 6 tons of shell has driven baseflow pH up 0.75 pH units. The normal pH for this stream is often in the 4's. The effect of the shells is immediate. It took about two hours to apply the shells by hand. By the time the shells were spread, the pH was already 0.75 pH units higher. A rainstorm two days later drove pH down and it took almost a month for the pH to recover. Even during the rainy weather following August 2, there is one-fifth to one-tenth the amount of acidity that is being delivered downstream (0.5-1.0 pH unit increase). This un-named perennial stream does not have fish, but does seasonally support wood frog tadpoles. Wood frogs are among the most acid tolerant of any aquatic vertebrates (Tome & Pough, 1982).

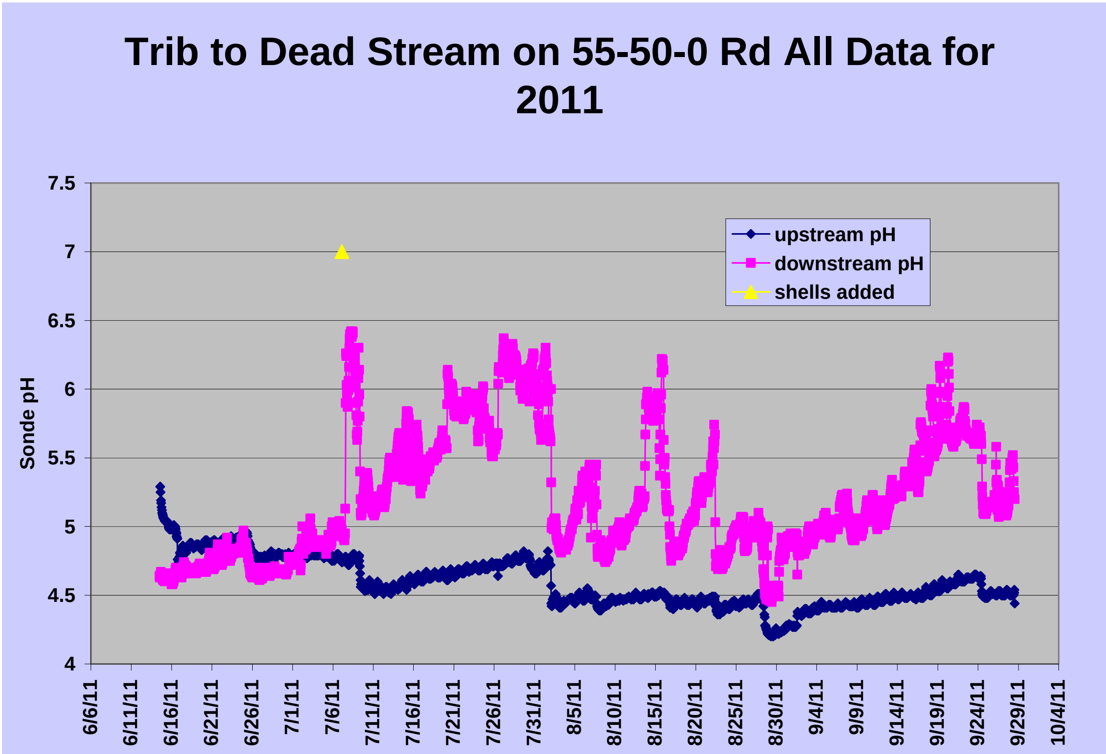

Figure 6. The sonde pH records above and below the shell application site (Site 3) on the un-named tributary on the 55-50-0 Rd are superimposed. A comparison of the records shows that the pH improvement can be as much as 0.75 pH unit during baseflow and is often 0.5 pH unit higher even during high flows.

Bowles Lake Stream maintains good water quality except during the highest flows (Figure 7). Site 2 was the last to receive shells in 2011. After 2 metric tons of shells were added on July 20, pH increased approximately 0.5 pH unit. This is the same amount of improvement observed at Site1 when 2 tons of shells were added for the first time the previous summer. Even during high flows, the pH has improved approximately 0.25 pH unit. The amount of time this stream spends below pH 6 is now measured in hours instead of days.

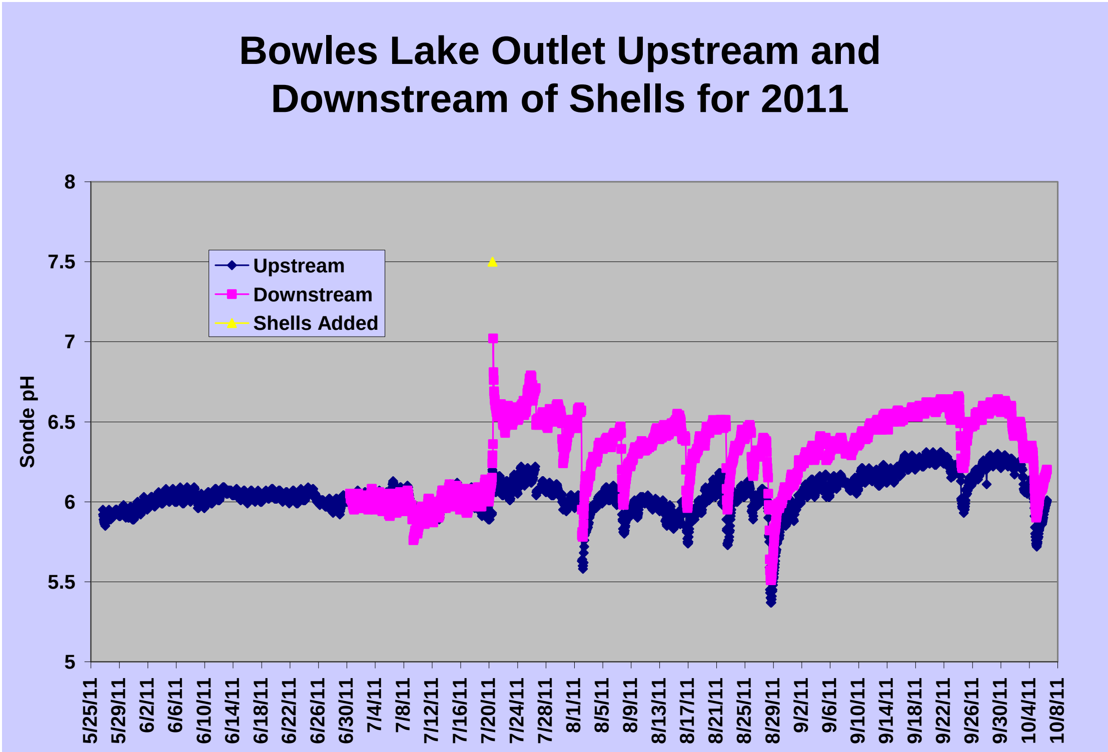

Figure 7. A comparison of before/after and upstream/downstream sonde pH records at Site 2 Bowles Lake Stream. Two tons of shells were added on July 20, 2011.

The lab data reflect the water quality improvements noted above. Naturally a few grab samples cannot represent the variability seen in the hourly resolution of the sonde data. Still, stream pH and dissolved calcium have increased and exchangeable aluminum has generally decreased (Table 2). The improvements are stream and location specific. Dead Stream at the 55-00-0 Rd experienced an increase of 0.72 to 0.80 in lab pH, an increase in calcium of 0.67 - 0.92 mg/L, and exchangeable Al decreased as much as 19 - 36 ug/L in different flow regimes. Calcium levels are approaching 4 mg/L below Site 1 which is the threshold for adult brook trout (Brocksen & Olem 1992). In experiments where brook trout life stages were evaluated in 2 mg/L and 5 mg/L Ca water, there was no difference in the survival of fingerlings and adults, but eggs and fry survival improved +15% and +55% respectively in the higher calcium concentration (Brocksen & Olem 1992). So in order to protect all life stages of brook trout, calcium levels should be at least 4 mg/L, and preferably at least 5 mg/L. Brook trout are the most acid tolerant fish in Maine waters, and very little is known about the calcium needs of other species. In order to have the best conditions for the most number of species, fishery managers need to consider pH, aluminum *and* calcium tolerances.

Bowles Lake Stream had the best initial water chemistry. In spite of recurrent storms in late summer and fall, Bowles Lake Stream pH increased at least 0.19 - 0.38 pH units, calcium increased 0.52 - 0.65 mg/L, and exchangeable Al which was already at low background levels (12-19 ug/L) stayed about the same. The unnamed tributary had the worst initial water chemistry. For this tributary, lab pH improved 0.41 - 0.46 units during strong flows, calcium increased more than one mg/L (1.02 - 1.06 mg/L) and exchangeable Al remained about the same in this low pH environment. In the lower watershed, it is not clear if the pH improved at the 58-00-0 Rd since there are different dates and flow regimes. However, calcium appears to have improved by 0.52 - 0.75 mg/L in spite of the dilution effects of strong rain storms. Exchangeable aluminum was still at harmful levels for fish during high flows and calcium levels remain critically low.

Table 2. A summary of lab data from the Sawyer Environmental Chemistry and Research Lab at the University of Maine is presented above. Pairs of Above/Below data from the shell application sites and unpaired Before/After shell additions in the lower watershed (at the 58-00-0 Rd) are color coded for easy comparison. The collections from 6/23/2001 for Bowles Lake Stream and the un-named tributary are not color coded because this date is prior to any shells being added.

Sample ID	Date	Flows	pH	Ca mg/L	Al x µg/L
Dead Str 55-00-0 Rd Above Site 1	**6/23/2011**	**Low**	5.99	2.10	32
Dead Str 55-00-0 Rd Above Site 1	**8/25/2011**	**High**	5.82	3.10	70
Dead Str 55-00-0 Rd Above Site 1	**10/13/2011**	**Medium**	5.73	3.15	55
Dead Str 55-34-0 Rd Below Site 1	**6/23/2011**	**Low**	6.71	3.02	13
Dead Str 55-34-0 Rd Below Site 1	**8/25/2011**	**High**	6.56	3.84	34
Dead Str 55-34-0 Rd Below Site 1	**10/13/2011**	**Medium**	6.53	3.82	24
Bowles L Str Above Site 2	**6/23/2011**	**Low**	6.18	1.06	19
Bowles L Str Above Site 2	**8/25/2011**	**High**	6.28	1.15	13
Bowles L Str Above Site 2	**10/13/2011**	**Medium**	6.24	1.25	13
Bowles L Str Below Site 2	**6/23/2011**	**Low**	6.23	1.07	12
Bowles L Str Below Site 2	**8/25/2011**	**High**	6.66	1.80	14
Bowles L Str Below Site 2	**10/13/2011**	**Medium**	6.43	1.77	13
Unnamed Trib Above Site 3	**6/23/2011**	**Low**	4.91	1.30	92
Unnamed Trib Above Site 3	**8/25/2011**	**High**	4.72	1.60	122
Unnamed Trib Above Site 3	**10/13/2011**	**Medium**	4.72	1.61	86
Unnamed Trib Below Site 3	**6/23/2011**	**Low**	4.98	1.26	112
Unnamed Trib Below Site 3	**8/25/2011**	**High**	5.13	2.62	109
Unnamed Trib Below Site 3	**10/13/2011**	**Medium**	5.18	2.67	86
Dead Str 58-00-0 Rd Before	**6/23/2011**	**Low**	6.28	1.85	45
Dead Str 58-00-0 Rd After	**8/25/2011**	**High**	5.82	2.36	88
Dead Str 58-00-0 Rd After	**10/13/2011**	**Medium**	6.04	2.60	43

Above and below comparisons of the lab data were tested for statistical significance using paired t-tests. For the 55-00-0 Rd, paired t-test results show that the increases from a mean pH of 5.85 to 6.60 ($p = 0.0010$, $df = 2$) and the increase in mean calcium from 2.78 to 3.56 mg/L were both highly significantly different ($p = 0.0091$). The differences in Alx was statistically significant at a lower level of certainty (52.33 decreased to 23.67 ug/L, $p = 0.0296$). For Bowles Lake Stream, due to the small sample size ($df = 1$) all of the means were not statistically different. At the un-named tributary, mean pH was significantly higher (4.72 increased to 5.55, $p = 0.0365$, $df = 1$) and the mean calcium concentrations are statistically higher (1.60 increased to 3.65 mg/L, $p = 0.0122$), but Alx is not statistically different. For the 58-00-0 Rd, an un-paired t-test is impossible because there is only one value in the Before category ($df = 0$).

The electrofishing results show increases in fish populations one year after the culvert replacements and more fish one year after the first shell application. At the 55-00-0

Road, within the 200 m study reach, fish numbers have improved and there are now have multiple year classes of brook trout (Figure 8). Brook trout are the most abundant fish and the only one that caught each year. Atlantic salmon were stocked as fry at the 55-00-0 Road in the spring of 2011, and were still abundant at the time of the electrofishing. Trout have increased from 34 fish per study reach found in 2007, to 13 in 2009, to 95 in 2010 (caught the same day the first shells were added, and thus a baseline survey), to 100 trout in 2011. In 2011, there are more of the older fish present, including second and third year fish. Clearly there is some year-to-year variation in species present and numbers of individuals. The culvert improvements might explain the increase in brook trout in 2010. The overall fish abundance has increased four-fold in 2011.

The increase in total fish numbers in 2011 is presumably due to a combination of factors. The salmon were stocked here as unfed fry in the spring of 2011, but it is reassuring that some of the fish stayed and survived. The new culverts allow fish of all kinds to move freely within the watershed. The improved water chemistry presumably is attractive to all species. Possibly the improved pH and calcium levels are attractive to fish. In the available literature, even for hatchery fish that get nutritionally balanced food, fish get their calcium requirements from ambient water through their gills (Danner 2004). Brook trout will seek out pH refugia during acidic storm episodes (Bulger et al 1995, Gerritsen et al 1996). Presumably salmon are also attuned to chemical signals and will seek better water quality.

Small changes in pH can make large differences in fish health. For instance, for Atlantic salmon pre-smolts (0+ parr) held in experimental channel-shaped tanks (Kroglund et al 2001) for 3 months at "mild acidity" pH 5.9 with "mild" Alx values below 25 ug/L, the fish had severe changes in gill morphology, elevated gill aluminum, elevated blood plasma glucose level, and reduced saltwater tolerance (but normal plasma chloride levels). These fish were able to recover completely in 9 days at pH 6.3 (but not at lower pH levels). This is the kind of sublethal effect that could cause fish to cluster at shell treatment areas.

With respect to the multiple year classes of brook trout observed in 2011, there is no way to know if the fish over-wintered here, or just seasonally utilized the treatment area. Brook trout are considered mature during their second summer. It will be interesting to see if there are second year salmon parr present in 2012.

Examination of the condition factor K shows a few individuals (1-3) each year that are in poor condition. Because each species has a different body shape, condition factors are compared only within each species, and only among the same age classes. In this case, only young-of-the-year (YOY) brook trout were available for comparison among years. Condition factor K has been of variable usefulness in other acidification studies. For instance, for blacknose dace in Shenandoah National Park streams, fish condition K was strongly affected by acidic exposure (but K was not a good indicator with other species, Bulger et al 1995). In Dead Stream, brook trout condition overall was good for all years. So far, like the Shenandoah study, there are no obvious trends in K for brook trout.

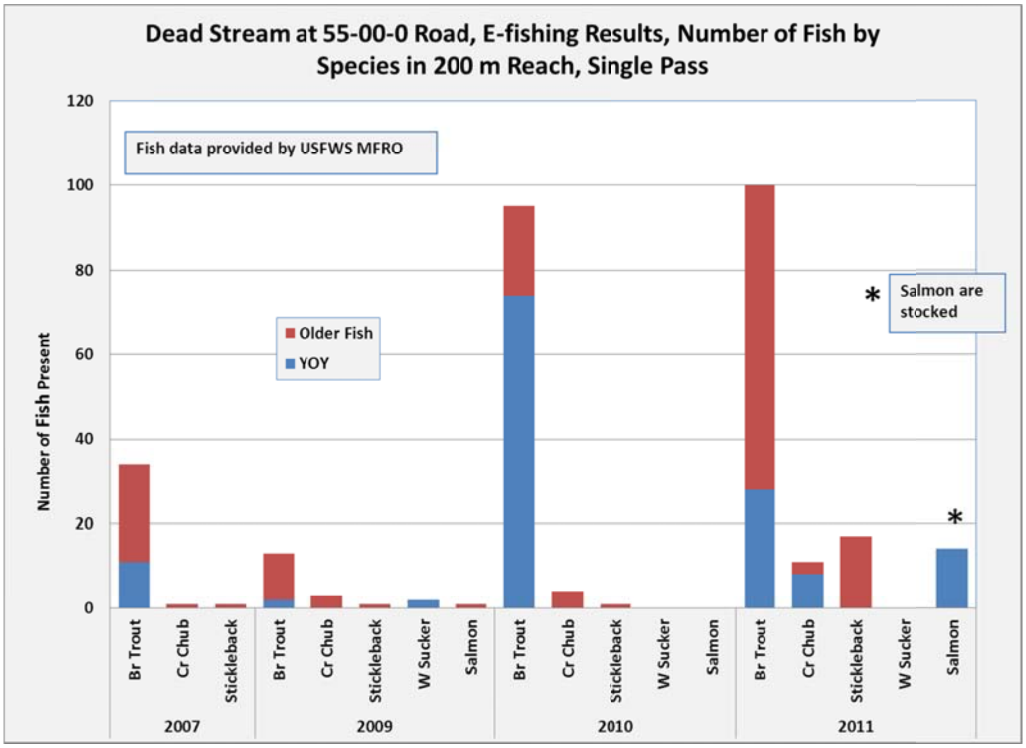

Figure 8. A summary of summer electrofishing results at the 55-00-0 Rd study reach by year. Both 2007 and 2009 represent the "Before" condition, 2010 is after road crossings were up-graded to fish-friendly culverts and "Before" shells were added, and 2011 represent one year after the first shells were added. A total of 36 fish were found in 2007, 21 were found in 2009, 100 were found in 2010, and 142 were found in 2011 within the 200 m study reach. More multiple year classes were observed for brook trout in 2011. Salmon fry were stocked below the 55-00-0 Rd in the spring of 2011.

Species diversity has varied over the years from 3 species in 2007, to 4 species in 2009, to 3 in 2010, and back to 4 species in 2011. The diversity has probably not changed, but there is a large year-to-year variation. However, the number of total fish has increased greatly so that the chance of seeing a fish of a given species has increased (species representation has improved). Streams with low numbers of individuals and low species diversity are among signs of acid stress and would be expected to respond to liming. However, recovery takes time; and many years of experience of liming rivers in Norway shows that restorations can take 8-12 years or more for fish (Hesthagen & Larsen 2003).

Sometimes when only brook trout are present, or if year classes are missing, that can be an indication that a stream is experiencing episodic acidification. Less hardy fish are killed or they move out of the watershed. Dead Stream is subjected to acidic episodes, but it is impossible to know if the absence of fish species in any one year is due to an event. Cause and effect are very difficult to establish with any degree of certainty in natural habitats.

At Bowles Stream, electrofishing in 2010 (the baseline) produced no fish. However, two fish, possibly creek chubs were observed while tending sondes. In the summer of 2011, unidentified minnows were rarely observed. After the shells were introduced, fish observations have continued, but are still rare. Electrofishing results in 2011 found two creek chub and a juvenile chain pickerel in a 100 m study reach (pickerel are normally a lake species, probably from Bowles Lake, but a species that uses streams for dispersal). It is curious that the stream with the best water chemistry has few fish. Possibly, the extreme chemistry of the un-named tributary, may act as a barrier to fish movement within Bowles Lake Stream. Warren et al (2008) attribute similar chemical barriers in Hubbard Brook (New Hampshire) as a factor in determining the present distribution and diversity of fish in that watershed.

There are other projects going on in the Dead-Bowles Lake Stream watershed and this complicates the interpretation of fish distributions and numbers. Fish can now move more freely within the watershed through improved culverts. They can find better forage areas, escape from summer heat or acidity, and find better spawning areas. This would be expected to produce the same kind of results seen from better water quality. Another complication was that Atlantic salmon are stocked in this watershed below the 58-00-0 Rd and fry were also stocked at the 55-00-0 Rd in the spring of 2011. Maine Dept of Marine Resources and Project SHARE are adding coarse woody debris to a site below the 58-00-0 Rd. This would provide pools and habitat complexity for larger fish. As a result, improved fish abundance cannot be credited among the specific water quality and habitat improvements. It can only be said that all the improvements together have been successful.

The Izaac Walton Save-our-Streams methodology was designed for volunteer monitoring of macroinvertebrates. It was useful because it gave immediate feedback on invertebrate communities during the liming process. However, it also means that the available macroinvertebrate results are extremely preliminary. Collections so far suggest an improvement in the abundance of the good water quality indicators, i.e.

mayflies, stoneflies, and caddisflies and in overall "species" diversity. The mayflies appear to have experienced the biggest improvements.

Snails and "fingernail clams" were found for the first time in the 2011 collections below the Site 1 shell treatment. Two small snails and one freshwater clam were also found, but so far, this is a very small sample size. Mollusks have the same dependency on calcium carbonate as a structural component of their skeletons as do vertebrates. Snails and clams have the additional problem that the skeleton is exterior and can be exposed to erosive acidic waters. In the well-buffered marine environment, a change from pH 8.1 to 7.9 is enough to reduce the survival of clams and shellfish by crossing a threshold from a carbonate depositional environment to an erosive one (EPA Climate Change Science website). Freshwater clams and snails are found in a greater range of pH conditions, but are among the most sensitive animal groups to acidification (Jenkins et al 2005). So far, crayfish, amphipods and isopods (all Crustaceans) have not been observed in the Dead Stream watershed.

In fourty-one headwater streams in northeastern France with different levels of impairment due to acid rain, the acidic streams had no Crustaceans, Mollusks or mayflies (Guerold et al 2000). In Adirondack streams, acidic communities had half as many species as mildly acidic ones, and were dominated by stoneflies (56-86% of all individuals) with some blackflies, midges, and caddisflies (Simpson et al 1983). In experimental streams in the California Sierra Nevada, single acid pulses of pH 5.2 or 4.6 lasting for 8 hours could dramatically depopulate macroinvertebrate communities of their dominant species (the mayflies *Baetis sp* and *Epeorus grandis*) (Kratz et al 1994). The losses were due to both drift and mortality, and most of the drifting individuals were dead. A second acid pulse two weeks later had very little effect on invertebrate density. One advantage that some invertebrates have over fish, is the shorter life cycles (some invertebrates have multiple life cycles per year) which allows them to recover in a few years (1-3) if water chemistry remains stable (Lepori et al 2003). On the other hand, if streams are still experiencing acidic episodes, then recovery may be only partial (Clayton et al 1992).

Algal communities have also improved (Table 4). In 2008 and 2009 algal blooms (Figure 10) were noticed in Dead Stream at the 55-00-0 Rd and in the un-named tributary at the 55-50-0 Rd. No blooms have been observed at Bowles Lake Stream, possibly due to the deep shade or because this site had the best initial water chemistry. Blooms were formed by the green alga *Mougeotia* sp and long ribbons (about 1 m) of chain-forming diatoms (*Eunotia pectinalis* and *Fragilariforma virescens*). The two dominant diatoms represented 95% of all individual valves (one-half of the shell) present in one Dead Stream (Site 1) collection in July 2009 (12 total taxa present). Both of these diatoms are acidophils (Lampman et al 2008) and have been observed forming blooms elsewhere within the watershed (Figure 10). The relatively low species diversity, high dominance and algal blooms are common in disturbed streams and are not expected of undisturbed salmon streams. In 2011, one year after the first shell application, *Mougeotia* was reduced to occasional tufts and diatoms were reduced to felts on rocks. On the post-treatment diatom slides, there were 61 taxa, and the two dominant species (*Fragilaria capucina var. rumpens* and *Eunotia soleirolii*) represented 32% of the diatoms present. The original two bloom formers were reduced to 2% and 4% respectively in the most recent counts. *Fragilaria capuchina var. rumpens* is typical of circumneutral to alkaline water, but like circumneutral *Eunotia soleirolii* will occur in mildly acidic waters. The mean number of species has increased from 18 to 58 and the mean number of genera from 9 to 25. The differences in these means are highly significant (unpaired t-test, $p < 0.001$, $df = 3$). In other words, the diatom flora now looks more like a normal trout stream. There has not been a repeat of the bloom conditions.

Table 3. Summary of species diversity trends in diatom communities at Dead Stream at the 55-00-0 Road. The number of individuals identified and counted is N, the number of species (Spp) and genera are provided for each sample date. The standard effort is that N should be 500-600 individuals (but since the tallies are done by hand, the final score varies).

Diatom Analysis - Dead Stream at 55-00-0 Rd

	Before			After	
	Jun-09	Jul-09	Sep-09	Aug-11	Aug-11
N	637	486	641	749	601
Spp	12	18	23	61	55
Genera	8	9	10	24	25

Figure 10. Photograph of an algal bloom on the Machias River at the Wigwams. This bloom is a combination of two diatom species, *Eunotia pecti*nalis and *Fragilariforma virescens*. Blooms like this photo have also been observed in Crooked River at the 52-00-0 Rd, the Narraguagus River in Cherryfield in the Cable Pool, and the Pleasant River at Saco Falls. Blooms in Dead – Bowles Lake Stream were never this bad. Blooms in Dead Stream (Site 1) were about one-half the biomass seen in this photo, while blooms at the 55-50-0 Rd (Site 3) were approximately one-tenth this biomass. No blooms have been observed in Bowles Lake Stream (Site 2).

In Europe, the Eastern United States and eastern Canada, algal mats and blooms are common in acidified streams and lakes (Hendrey 1982, Lampman et al 2008). The dominant species often include *Mougeotia* and *Fragilariforma virescens*, two of the three dominant species found in Dead Stream blooms. In literature reviews, algal blooms typically disappear after liming (Hendrey 1982). In contrast to fish communities, which may take 10-20 years to be fully restored, or to macroinvertebrates which may take 1-3 years or more, diatoms adjust within 1-2 weeks to ambient conditions (Lepori et al 2003, Hirst et al 2004).

IV. Conclusions:

The clam shell-liming program has three goals, to (1.) moderate pH, (2.) reduce toxic aluminum, and (3.) provide enough calcium to support diverse aquatic communities. The water quality of the Dead Stream watershed has improved, and was noticeable within 60 minutes of shell applications. Stream pH is higher and exchangeable aluminum is lower. However, both pH and aluminum can still reach harmful levels during high flows. Many storm episodes are still too acidic for smolting salmon (the pH should be at least 6.4 from the pre-smolt period starting February 1 to the end of the smolt run around July1, Staurnes et al 1995). The original goal was about pH 7, and probably can be scaled down to something around 6.8, provided a pH of 6.4 is achieved during high flows in the spring. Dissolved calcium levels have improved even in high flow conditions and even at sites farthest from the clam shells. Calcium levels are around 2-4 mg/L, but should be at least 4 mg/L. So far, the dose calculations are conservative for clam shells and Maine streams. There should be additional benefits from a higher dose. Next year, a double dose will be tried in one new experimental stream. Nearby Canaan Stream (on the 59-00-0 logging road) is suitable, since it is small and the effects can be isolated within this watershed (it is a very small stream in comparison to Old Stream).

The fish in Dead Stream are doing better, but are likely to be responding to all of the improvements in the watershed. These other improvements complicate the interpretation of the better water quality. By expanding to other sites, such as Honeymoon Brook Canaan Stream and First Lake Stream, a baseline can be established after more time has passed since culverts were replaced in 2009.

Dead Stream also has more diverse algal and macroinvertebrate communities. Summer algal blooms which were once common have not been observed since the first shell treatments. Among the macroinvertebrates, the clean water indicators have become more numerous. Mayflies are especially good indicators of acidification (Petrin et al 2007, Moe et al 2010). For instance, of 77 mayfly species with known environmental requirements only 4 species are acidobiontic (occur in acidic waters below pH 6.0) (Hubbard & Peters 1978). Because macroinvertebrates are so important, professional invertebrate assessments are planned for the 2012 field season.

Liming projects have usually been successful and any negative consequences are rare (Olem et al 1991, Brockson & Olem 1992). In Norway and Sweden, Atlantic salmon and brown trout fisheries have been restored by adding agricultural lime to headwater lakes (the lime is distributed on top of winter ice and then allowed to melt into the lake in the spring) or rivers are limed by mechanical dosers with computerized controls (Kroglund et al 2001a, Sandoy & Langaker 2001, and Norrgren et al 1993). The target for salmon is generally around pH 6.2, but is increased to at least 6.4 during the pre-smolt period up to the smolt run (i.e., from February 1 through July 1, Staurnes et al 1995, Kroglund et al 2001b). Amphipods, tadpole shrimp and gastropods are important food resources for brown trout, but are absent from acidified streams. After 6 years of liming, Svartavatnet Lake watershed (Norway) brown trout are recovering but important invertebrates are lagging (Fjellheim et al 2001). In spite of stocking the amphipod *Gammarus* and the snail *Lepidurus arcticus*, these species are not found in the lake littoral benthic surveys. However, these species and some related species have been found in trout stomachs. These species appear to have survived in refugia and are thriving in microhabitats within the lake or in tributary streams. The recovery of biodiversity from refugia is a recurrent theme in the acid rain literature.

The main negative aspects of liming are: (1.) the cost, (2.) the fact that the treatment has to be reapplied periodically, and (3.) in limed rivers, the mixing with untreated tributaries results in a potentially deadly mixing zone. While the aluminum is being converted to less toxic species by the change in pH, it takes some time to be completely changed (Kroglund et al 2001c). While the changes are occurring, some forms of the aluminum are more toxic than the original Alx (Poleo et al 1993, Rosseland & Hindar 1991). These problems can be minimized by using clam shells which are relatively cheap and low-tech, and dissolve slowly (so there are no sudden changes in pH) and by using multiple treatments locations (so that tributaries are also treated). Thus, the downstream reaches all the way to river mainstem have some treatment. The mainstems of rivers are typically better off than their acidic tributaries (Lawrence 2002). In Old Stream, the mainstem below First Lake is circumneutral, and therefore not at risk from mixing zones.

Just like fish use thermal refugia to escape summer heat, fish exposed to episodic acidification will seek out water quality refugia (Gerritsen et al 1991, Bulger et al 1995, Gensemer & Playle 2010). Between acidic episodes, sensitive species can venture into parts of the watershed where they cannot survive long-term (Baldigo & Lawrence 2001). These refugia occur to some extent naturally. Marine-derived and carbonate-rich bedrock in the Lanpher Brook watershed is a natural source of buffering capacity. However, Washington County is dominated by silica-rich and nutrient-poor granite bedrock, so well-buffered refugia are rare. The plan is to create more of these safe places for fish. If enough headwaters are treated, then even the larger rivers will have better water chemistry. With good initial results in Dead Stream, this is probably a good time to expand the experiment to Honeymoon Brook, First Lake Stream, and Canaan Brook.

V. Plans for Next Year

The experimental permit runs through November of 2014, so there are three more field seasons. The original permit application proposed that if the experiment went well at Dead Stream, that it would be expanded to other streams. The plan is to continue shell applications at Dead Stream and expand to a tributary to Honeymoon Brook, First Lake Stream, and Canaan Brook in the 2012 field season. Thus, it can be determined if the initial success can be replicated at other sites. Canaan Brook will receive a double dose (as recommended by the state liming program in West Virginia for all beginning projects). Macroinvertebrates will be sampled in summer and be professionally assessed.

Honeymoon Brook had fish kills during the summers of 2008 and 2011 after heavy rains following dry spells. In the summer of 2008 dead young-of-the-year brook trout were observed only in the un-named tributary. In 2011 on the mainstem of Honeymoon there were dead YOY trout, creek chub and other minnows after strong August storms. The pH of this stream has been observed in the low 5's with Alx around 100 ug/L after fish kills. The plan for Honeymoon Brook is to begin with shell applications in a tributary where we saw the 2008 fish kill. If things go well, we hope to expand shell applications in the summer of 2013 to the mainstem above where Atlantic salmon are stocked and in some headwater access points.

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