FINAL REPORT

Restoration of Spawning Habitat for Trout in Big Spring Creek, Cumberland County, Pennsylvania

Submitted to:
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INTRODUCTION

Big Spring Creek, south-central Pennsylvania, once supported a dense brook trout population according to state natural resource professionals. Over the past 50 years, wild brook trout have nearly disappeared from this limestone stream, and those remaining now share habitat with hatchery-reared brown trout and rainbow trout and naturalized brown trout and rainbow trout. State resource agencies and private conservation groups are strongly in favor of restoring native brook trout to this stream. A number of changes have occurred in Big Spring Creek that, singly or in combination, may have contributed to the loss of wild brook trout. A state fish hatchery was constructed next to Big Spring, which is the sole source of water to the stream's headwaters. In the past, a commercial fish hatchery also operated in the vicinity. The channel morphology has been altered by old mill dams, which have been largely removed, though remnants of these structures continue to influence flow patterns. There is ample evidence of stream bank erosion and an absence of hiding cover for adult trout. The stream substrate is embedded (Embeck 2000) or compacted (Black and Macri 1997), and it is apparent that trout would have difficulty displacing these sediments during spawning.

The Pennsylvania Fish and Boat Commission (PFBC) is prepared to commit substantial funds to restore the physical habitat of Big Spring, but to do so, they need to know what factors are responsible for the loss of spawning habitat. Several potential causes have been identified as focuses for this investigation. **(1) Changes in surface water quality**. Water quality in the creek might have changed to conditions that are less suitable for trout due to a change in the quality or quantity of water arising from the spring source, direct effects of hatchery effluent discharge, and/or changes in management of the creek flows. **(2) Physical effects of substrate consolidation.** Substrate consolidation (embeddedness) might be preventing fish from successfully constructing redds by interfering with their ability to move substrate material. Substrate consolidation might be the result of one or more causes, including accumulation of fine material in interstitial spaces in gravel (embedding, a physical effect of particle size), or consolidation of substrate due to calcium carbonate precipitation, phosphate cementation, or biofilm accumulation. Potential sources of fines include particulate material from the spring source, fish waste in the hatchery effluent, and material eroded from the creek banks. Calcium carbonate precipitation and phosphate cementation might result from water quality factors associated with the spring source, surface runoff, hatchery effluent, and/or creek flow management. **(3) Decline in interstitial water quality.** Substrate consolidation may be causing decreased substrate permeability so that oxygenated surface water cannot penetrate the gravels and/or accumulation of organic material might be causing a reduction in the quality of water in interstitial spaces in spawning substrates by increasing biochemical oxygen demand. Particular concerns for developing trout eggs are decreased dissolved oxygen and toxic levels of ammonia. To investigate these factors, the following objectives were identified.

Objectives

(1) Collect substrates from trout redds to characterize texture of spawning gravels.
(2) Use scanning electron microscopy techniques (SEM) to evaluate the likelihood that a biofilm or biogenic calcite deposition contribute to the embeddedness or consolidation of the stream substrate.
(3) Measure dissolved oxygen (DO), ammonia, and other water quality parameters in surface water and stream substrate interstitial water.
(4) Document the locations and extent of trout spawning in Big Spring Creek.
(5) Attempt to identify the source(s) of fine material accumulating in interstitial spaces in the stream substrate and on the surface of the substrate in slow-moving portions of the stream.

METHODS

Sampling sites

Big Spring Creek is a low-gradient limestone stream originating from a spring water source and flowing north. Historically (since the 1970s), much of the flow originating from the springs was diverted to the Big Spring Fish Culture Station (hatchery), which was operated by PFBC. The treated effluent discharge of the hatchery constituted all or most of the flow of the creek downstream of the hatchery, since there are no other sources of water to the stream. At the onset of this study, one of the small spring flows was allowed to bypass the hatchery to flow directly to the creek, but it comprised a small proportion of the total spring water flow. A description of the sampling sites on Big Spring Creek is provided in Table 1 and Figure 1 (map). Sites on Big Spring Creek are labeled BS0 through BS3. Site BS0 is located near the spring source and upstream of the fish hatchery effluent discharge. Sites BS1 through BS3 occur at increasing distances downstream from the hatchery effluent discharge.

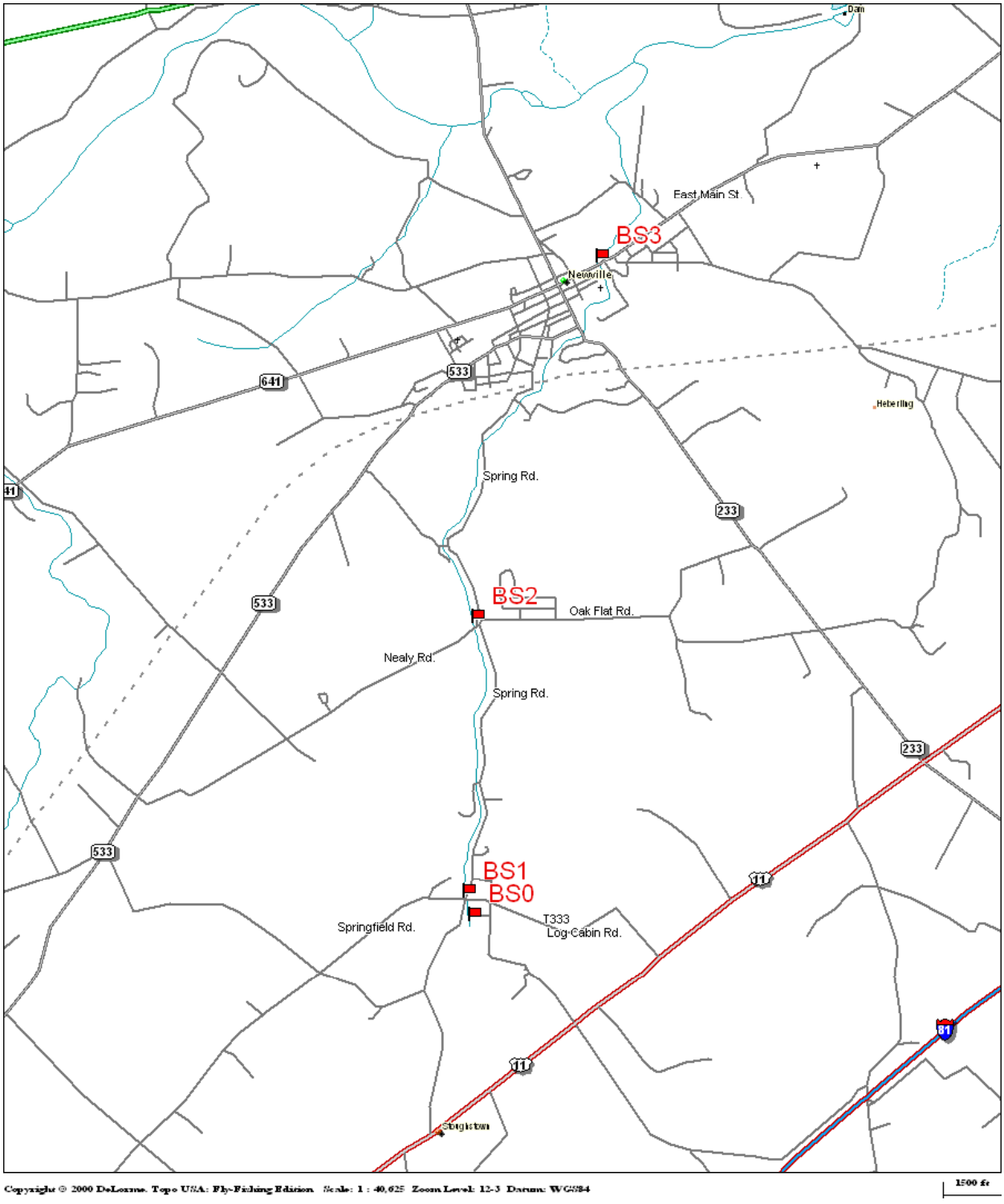
Letort Spring Run, near Carlisle, PA, was selected as a reference stream because of its geographic proximity to Big Spring Creek and because it exhibits some characteristics that make it comparable (limestone stream, arises from a spring source, more similar in flow and gradient than other nearby streams). Letort Spring Run has a healthy spawning population of trout and thus is considered to be representative of conditions that might be goals for restoration projects on Big Spring Creek. Sampling sites on Letort Spring Run are described in Table 1.

Table 1. PACFWRU sampling sites on Big Spring Creek and Letort Spring Run.

Site	Description
BS0	Area where the Big Spring emerges from the ground at the head of Big Spring Creek, prior to diversion to the Big Spring Fish Culture Station. No apparent substrate consolidation. Rocky bottom covered with fine silt that appears to originate from the spring or from decay of plant material.*
BS1	Downstream of the fish hatchery effluent discharge. Sampling sites were just downstream of the remains of the old mill dam at the lower end of "the ditch" or just downstream of Spring Road Bridge. Substrate was consolidated. Previous work conducted by Black and Macri (1997) indicates that the benthic invertebrate community was negatively impacted by organic material (compare to their site BSC02), as indicated by Hilsenhoff biotic indices. Our own qualitative assessment based on professional judgment revealed an abnormally great biomass of isopods, which also suggests organic contamination.
BS2	Further downstream from the hatchery discharge than BS1, near the point where Nealy Road crosses Big Spring Creek. Moderate substrate consolidation. In our opinion, benthic invertebrates still appear to be negatively impacted. Black and Macri (1997) reported negatively impacted invertebrate communities, based on Hilsenhoff biotic indices, at sites near this one.
BS3	Site most distant (downstream) from the hatchery discharge, downstream of BS2 on Big Spring Creek and just downstream of the Laughlin Grist Mill in Newville, PA. Little or no substrate consolidation. Based on our informal qualitative assessment, the condition of the benthic invertebrate community is better than that observed at BS1 and BS2. Likewise, Black and Macri (1997) reported improvement of the invertebrate community at their site BSC04 (downstream of the Laughlin Grist Mill dam) relative to upstream sites.
LE0	Downstream of the head springs on Letort Spring Run, just upstream of the point where Bonnybrook Road crosses the stream.*
LE1	Downstream of LE0 on Letort Spring Run, just downstream of the footbridge across from the quarry, near the intersection of One Way Road and Bonnybrook Road.*

* The condition of the invertebrate community at this location was not assessed in this study and was not reported in the 1997 study by Black and Macri.

Figure 1. Map of sampling sites in Big Spring Creek.



Timeline

Field work and sample collection for this project commenced at the end of June 2001. Dates related to sampling and other activities are provided in Figure 2 and will be identified in the relevant sections that follow. Hatchery production was phased down in fall of 2001, and all fish from the Big Spring Fish Culture Station were stocked out by 5 November due to concerns about potential effects of the hatchery effluent discharge on Big Spring Creek (J. Arway, PFBC, personal communication). Therefore, results from samples collected past 5 November, in particular, might not be representative of conditions in previous years.

Figure 2. Timeline of important events, including sampling events.

27–28 June 2001	Surface water quality
10 September 2001	Interstitial water sampling probes deployed
3 October 2001	Surface water quality, interstitial water quality (syringe sampling), precipitation modeling
30 October 2001	Surface water quality, interstitial water quality, redd survey
5 November 2001	Hatchery no longer contained fish
6 and 9 November 2001	Substrates collected for particle characterization, redd survey
5 December 2001	Surface water quality, interstitial water quality
10 December 2001	Substrates collected for particle characterization
24 January 2002	Surface water quality, interstitial water quality, redd excavation
27 February 2002	Saturometer reading, redd excavation

Surface water quality and substrate interstitial water quality

Water sample measurements and sample collections for general water quality were made on 27-28 June 2001, 3 October 2001, 30 October 2001, 5 December 2001, and 24 January 2002. DO and temperature were measured with YSI DO meters (YSI Model 95 and YSI Model 58). In most cases, DO measurements were taken with both meters and the average was reported. Measurements of pH were made with the Corning

CHEKMITE® pH-15 Sensor, Oakton pHTestr 3™ with ATC (Forestry Suppliers, Jackson, MS) or with an Oakton pH300 portable pH/mV/°C meter (Forestry Suppliers, Jackson, MS). Oxidation-reduction potential (ORP, or redox potential) was measured with an ORP monitor (ORP3; Aquatic Ecosystems, Apopka, FL). All water quality instruments were calibrated in the field on the days they were used, except for the ORP monitor, which does not require calibration. Samples were submitted to the Water Quality Laboratory at the Environmental Resources Research Institute at PSU for analysis of total ammonia, total phosphorus, dissolved organic carbon (DOC), total organic carbon (TOC), alkalinity, nitrite, calcium, and magnesium. Un-ionized ammonia was calculated using methods described previously (Thurston *et al.* 1979). Ammonia values were not corrected for salinity or total dissolved solids. For samples with a method detection limit (MDL) less than 0.006 mg/L, un-ionized ammonia concentrations were not calculated because levels are too low to be toxicologically significant. Particulate organic carbon (POC) was calculated as POC = TOC – DOC. Total hardness was calculated from measurements of calcium and magnesium according to methods described previously (APHA 1995).

Four water samples were collected from Big Spring Creek (one from each site) on 28 June 2001, for analysis of biochemical oxygen demand (BOD_5). Samples were collected in 250-mL glass BOD sampling bottles and transported on ice to the analytical laboratory. BOD_5 was analyzed by Centre Analytical Laboratories, Inc. (now Exygen Research), State College, PA, using EPA Method 405.1: Biochemical Oxygen Demand, BOD (5 day, 20 °C).

In method similar to one used previously (Beard 1990), a set of interstitial water samples was collected on 28 June 2001, with a large blunt end stainless steel needle (Fisher Scientific; 14G × 6 in.) fitted to a 60 mL Beckton-Dickson plastic syringe with Luer-lock fitting (Fisher Scientific). To sample substrate interstitial water, a small piece of wire was inserted into the needle bore to keep material from plugging the needle. The entire length of the needle was inserted into the substrate, the wire was removed, the syringe was fitted to the needle by means of a Luer-lock fitting, and a sample of interstitial water was drawn into the syringe. A 60 mL portion of the interstitial water was placed into a clean beaker for immediate measurement of temperature, DO, ORP, and pH. Additional samples were withdrawn and transferred to a 500-mL plastic bottle for measurement of other water quality parameters. All interstitial water samples collected subsequent to June 28 were collected from sampling probes as described below.

Interstitial water sampling probes were installed on 10 September 2001 to monitor DO concentrations in substrate interstitial water. The interstitial water sampling probe for site BS1 was installed just downstream of Spring Road Bridge, the concrete bridge that allows Spring Road to cross the creek. Embeck (2000) suggested a sampling probe design (Maret *et al.* 1993), which was suitable with some modifications. Each sampling probe was constructed with a 21-inch length of 2-inch inner diameter (ID) schedule 40 PVC pipe. Holes were drilled in the pipe at regular intervals, with 6 holes around the circumference and 20 holes along each row along the length (120 holes per sampler). Holes were 7 mm in diameter. The entire pipe was covered with 150 micron nylon mesh

(Aquatic Ecosystems, Apopka, FL). A length of rigid clear acrylic tubing (Aquatic Ecosystems, Apopka, FL; 3/8-inch outer diameter (OD), 5/16-inch ID, drilled with 6 holes spiraled along its length) was suspended in the center of the interior of each pipe by gluing it to clean-out plugs fitted to each end of the pipe with 2-inch schedule 40 female adapters. Threads were sealed with Marine Goop contact adhesive and sealant. One end of the interior rigid tubing (approximately 1.5-inch length) extended through a hole drilled in the clean-out plug on one end of the PVC pipe. Marine Goop was used to create a seal between the protruding tubing and the plug. The protruding end of the interior tubing was fitted tightly inside the end of a 30-inch length of flexible 3/8-inch ID Tygon tubing (Aquatic Ecosystems, Apopka, FL). A tubing clamp (pinch type, Aquatic Ecosystems, Apopka, FL) was placed on the end of the Tygon tubing to prevent water and debris from entering. One sampling probe was installed at each site listed in Table 1. The sampling probes were buried at a depth of approximately 13 cm (typical depth for trout eggs to be buried in a redd) at locations within each sampling site that were considered to be most suitable for trout spawning, based on observations of flow, depth, and substrate composition. Brown trout and rainbow trout may bury their eggs at somewhat greater depths, with most eggs located 20 cm (7.9 in.) from the surface of the gravel (Chapman 1988). Smaller fish tend to construct smaller redds and to bury their eggs at lesser depths (Chapman 1988). In a stream in Ontario, all brook trout eggs were found at 16 – 20 cm depth in the substrate (Snucins *et al.* 1992). In a study of southwestern Ontario streams, Witzel and MacCrimmon (1983) found that most brown trout eggs were deposited at depths greater than 14 cm (5.5 in.) in the substrate, while brook trout eggs rarely were found at depths greater than 14 cm. The base of the egg pocket in brook trout redds in a southeastern Wyoming stream averaged 8.4 cm (range 5.5 – 12 cm) below the streambed surface (Young *et al.* 1989).

Water samples were collected from the sampling probes with a peristaltic tubing pump (Cole-Parmer, Vernon Hills, IL) consisting of a Masterflex 12V DC-powered drive (Model 7533-40) and a Masterflex EasyLoad LS pump head (Model 7518-12) and using Masterflex LS-15 Tygon lab tubing. The tubing attached to the sampling probe was joined to the tubing on the pump by means of a plastic hose-barbed quick-disconnect fitting (Aquatic Ecoystems, Apopka, FL). The connection point was held above the surface of the water to prevent surface water from entering the tubing. A volume of 1 liter of water, the void volume of the probe, was withdrawn from the sampling probe and discarded. This practice also flushed the tubing of water from the previous sampling site. The next 1 liter of water collected, presumably drawn from the surrounding substrate interstitial spaces, was collected for analysis of water quality parameters. At the end of a sampling day, the Masterflex tubing was thoroughly flushed with deionized water and allowed to dry completely. During a sampling trip on October 3, it was observed that the sampling probes at BS0 and BS2 had been removed from the substrate; they were immediately re-installed. The sampling probe at LE1 could not be located. Another was constructed and re-installed on November 6.

A saturometer reading was taken at BS0 on 27 February 2002 to determine whether gas supersaturation might be a factor contributing to failure of trout to spawn in that area. The measurement was made with a Sweeney saturometer (Aquamatics, Story Creek, CT),

and calculations of dissolved gas pressures were performed using GASWORKS4.bas, a BASIC program written by Barnaby Watten (U.S. Geological Survey, Leetown Science Center, Kearneysville, WV) and executed in Microsoft® QuickBASIC Version 4.5 (Microsoft Corporation, Redmond, WA).

Microbial biofilm on substrates

Substrate samples were collected for examination by light microscopy and scanning electron microscopy (SEM) to identify any obvious overgrowth of biofilm associated with the fish hatchery effluent discharge that might be involved in substrate consolidation. The sites were sampled on 28 June 2001, in the following order, from first to last: BS3, BS2, BS1, BS0. The substrate sampling areas were selected by locating areas judged by Carline to be suitable for trout spawning on the basis of flow and substrate composition, without regard to the degree to which the substrate was consolidated. Two sampling areas were chosen within each site, except BS0, where only one area was selected. At sites BS1 and BS2, at least one area sampled within each site demonstrated some degree of consolidation. First, substrate samples were collected from the upper two inches of material in an area the size and shape of a circle of 20 cm in diameter. Personnel collecting the samples wore disposable nitrile gloves and stood downstream of the sampling area. Whenever possible, samples were collected directly into tubes, which were sealed underwater. Stainless steel trowels or spoons were used to manipulate samples into bags or tubes when necessary. Larger pieces of gravel would not fit into sampling bags and tubes, and microorganisms and calcite deposits are more likely to be associated with, and simpler to detect on, finer particles. Therefore, larger pieces of gravel were discarded prior to distributing the sample among containers. From each sampling area, the following subsamples were collected: one 2-5 g sample of substrate was fixed in a 20-mL vial of ice-chilled preservative (2.5 % glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4)(Electron Microscopy Sciences, Fort Washington, PA); one 50-mL sterile polypropylene tube was filled approximately two-thirds full; four 5-10 g samples of substrate were placed into individual sterile Whirl-pak bags. After sampling the surface of the substrate in a sampling area, the area was cleared of substrate to a depth of approximately 5 inches. The trowel, spoon, and gloves were sprayed with a solution of 70% ethanol and rinsed with stream water prior to the next use to prevent cross-contamination of microorganisms among samples. Another set of samples was collected at 5-inch depth and handled in the same fashion as the surface samples. Between sampling areas, gloves were replaced with new ones and the trowel and spoon were sprayed with ethanol and allowed 10 minutes of contact prior to the next use. All samples were sealed in plastic bags and placed on wet ice immediately after collection. Samples were transported on wet ice to the laboratory on the day they were collected. Additional samples of substrate were collected on 9 July 2001, and preserved in glutaraldehyde buffer for examination of the biofilm; these samples were collected from areas of the stream substrate characterized as embedded or consolidated.

Heterotrophic plate counts

Fresh samples in the Whirl-Pak bags were taken immediately (on the day they were collected) to Dr. Mary Ann Bruns, Assistant Professor of Soil Microbial Ecology, PSU, for R2A heterotrophic plate counts. Microorganisms from one bag from each sampling location were cultured from fresh material, and the remaining replicate bags (3 per sampling area) were frozen at –80 °C. Heterotrophic plate counts were made for one surface and one subsurface sample from each of two sampling areas within each of the sampling sites, except for BS0, where only one area was sampled. One-gram samples were vortexed vigorously and for 2 min. in 0.1% peptone water to remove biofilms, which were diluted and spread-plated on R2A agar plates. Plates were incubated at 25 °C and counted after 2 and 10 days. Although the standard incubation time is 10 days, plates were also counted after 2 days because early rapid growth of a few spreading colonies (probably *Bacillus* spp.) was observed. These "spreaders" can affect outgrowth of other colonies and cause 10-day counts to be lowered artifactually (M. A. Bruns, PSU, personal communication).

Light microscopy

Substrate samples in 50-mL tubes held overnight in a refrigerator at 4 °C. The following morning, the samples were taken to Dr. Richard Unz, Professor of Civil Engineering / Environmental Microbiology, PSU, for a brief assessment of the microbial community using a light microscope.

Scanning electron microscopy

Samples preserved in glutaraldehyde buffer (see **Microbial biofilm on substrates**) were left in a refrigerator at 4 °C until preparation for examination of the biofilm by scanning electron microscopy (SEM). Two mL of fine particle suspension was drawn into a sterile syringe and filtered dry through a 0.2 micron track etched filter in a Swinnex syringe cartridge with gasket. Each filter was transferred to a specimen carrier (Electron Microscopy Sciences, Fort Washington, PA) inserted into a Costar 96-well flat bottom microplate, and samples were dehydrated by transfer through a series of ethanol washes at 4 °C as follows: 25% ethanol (EtOH) for 3 min, 50% EtOH for 3 min, 70% ethanol for 2.5 hr, 85% EtOH for 3 min, 95% EtOH for 3 min. Samples were further dried by supercritical fluid extraction in a BALTEC SCD030 critical point dryer (Techno Trade, Manchester, NH). This drying process is used to preserve the structure of cells in the biofilm rather than air-drying, which destroys cellular integrity. Dried sample was pressed onto carbon adhesive tabs on large aluminum mounts, then sputter-coated using a BALTEC SCD050 sputter-coater (Techno Trade, Manchester, NH). SEM examination was conducted with the assistance of Dr. Rosemary Walsh, Electron Microscopy Facilities for Life Sciences, PSU, using a JEOL JSM 5400 SEM (Peabody, MA) in SEI mode. Digital archiving was performed with a Princeton Gamma Tech (PGT) Integrated Microanalyzer for Imaging and X-Ray (IMIX-PC v.10, Princeton, NJ).

Accumulation and consolidation of fine material in substrates

Texture of stream substrate

Substrate samples were collected from trout redds (see **Distribution of trout redds at Big Spring Creek**) with a McNeil substrate sampler on 6 November 2001 to determine whether spawning gravel texture was controlling locations of redds and survival of embryos. On 10 December 2001, substrate samples were collected with a McNeil substrate sampler from all of the sites listed in Table 1 to determine whether texture of spawning gravels affected substrate interstitial water quality. Areas within sites were selected for sampling if they appeared to be suitable, on the basis of depth and flow, for trout spawning and were in close proximity to interstitial water sampling probes. All stream substrate samples were dried in a drying oven at 103-105 °C. A series of sieves (mesh sizes 12.7, 4.00, 2.38, 2.00, 1.68, 1.19, 1.00, 0.85, 0.495, 0.0625, and <0.0625 mm) was employed to sort dried samples in a geometric progression of 11 size-classes. Geometric mean particle size (GMPS, or d_g) for a sample was calculated by raising the grain size at the midpoint of each size-class to a power equal to the fraction of its weight expressed as a decimal, then multiplying the products for each class to obtain a final product (Lotspeich and Everest 1981). Sorting coefficient (S_o) was calculated by taking the square root of the quotient of the grain size at the 75th percentile divided by the grain size at the 25th percentile (Lotspeich and Everest 1981). Fredle index was calculated as d_g/S_o. The percentage of the total mass of each sediment sample composed of fines that pass through a sieve with a pore size of 1 mm (% fines < 1 mm) also was determined.

Calcite deposition in substrate

Samples of substrate were collected on 26 July 2001, as described previously (see ***Microbial biofilm on substrates***). These samples were collected from areas of the stream substrate characterized as embedded or consolidated. Samples were air-dried at room temperature and examined for evidence indicating calcite deposition due to secondary (biogenic and/or physical) processes, such as calcite crystals (silt size, 2 – 50 μm) overgrowing other particles or demonstrating a "honeycomb" morphology suggesting biogenic formation (Cicerone *et al.* 1999). Calcite particles arising from parent material (e.g., erosion of rock) tend to be larger than 50 μm and demonstrate morphologic characteristics of a well-cleaved particle (sharp etches, terraces, and corners) (Cicerone *et al.* 1999). SEM examination was conducted with the assistance of Dr. Rosemary Walsh, Electron Microscopy Facilities for Life Sciences, PSU, using a JEOL JSM 5400 SEM (Peabody, MA) in SEI mode. Digital archiving was performed with a Princeton Gamma Tech (PGT) Integrated Microanalyzer for Imaging and X-Ray (IMIX-PC v.10, Princeton, NJ). Additional SEM work was conducted with the assistance of Dr. Maria Klimkiewicz, MRL, PSU, using a Hitachi S-3500 N Variable Pressure PC-SEM (San Jose, CA). Attached to the SEM was a PGT PRISM Si(Li) detector with IMIX -PC Analyzer system, which was used for energy dispersive spectroscopy (EDS). The latter instrument made it possible to subject isolated particles viewed with SEM to EDS analysis to determine whether particles that appeared to exhibit morphology indicative of biogenic calcite deposition were actually composed of calcite.

At the suggestion of Dr. John Black, a simpler on-site assessment of calcite deposition was conducted at the sampling sites as follows. A small sample (10 – 20 g) of freshly collected stream substrate material (<1 mm size fraction) was placed into a petri dish. Stream water collected from the same site was immediately added so that the sample was barely covered. While the sample dish was observed under a dissecting microscope (4x power), 0.1N hydrochloric acid was added to the sample, one drop at a time. If calcium carbonate precipitate is present, carbon dioxide gas will evolve as the acid is added, producing bubbles on the surfaces of the grains.

Mineral precipitation modeling

PHREEQC Interactive 2.4.2 ALPHA (U.S. Geological Survey 2001) is a computer program that was used to determine the likelihood that surface water conditions in Big Spring Creek and Letort Spring Run were conducive to precipitation of calcium carbonate or other minerals. The acronym PHREEQC stands for "pH (pH), RE (redox), EQ (equilibrium), C (program written in C)." PHREEQC is designed to perform a variety of low-temperature aqueous geochemical calculations and is based on an ion-association aqueous model. It has capabilities for speciation and saturation-index calculations. The input consists of water quality data and element concentrations measured in water samples. Surface water samples for elemental analysis were collected from BS0, BS1, LE0, and LE1 on 3 October 2001. Samples were collected in clean 500-mL plastic bottles (pre-rinsed 6 times each with deionized water) and were transported on ice to the analytical laboratory. Samples were analyzed for the presence of certain anions by Scott Atkinson, MRL, PSU. Cations were analyzed by dc plasma emission spectrometry (Spectraspan III dcp, Spectrametrics Inc., Andover, MA.) conducted on unfiltered water samples. Water samples for anion analysis were filtered through a 0.2 micron filter (Teflon Acrodisc, Pall Corp., Ann Arbor, MI) to remove solids (>0.45 micron) and larger colloidal particles (0.001 to 0.45 micron), then analyzed by ion chromatography (Dionex Model 3010i, Dionex, Sunnyvale, CA). The necessary water quality measurements were made during surface water quality monitoring on 3 October 2001 (see above, **Surface water quality and substrate interstitial water quality**). Input data were provided to Dr. Barry Scheetz, MRL, PSU, who performed the calculations with PHREEQC.

Crystal structure of minerals in fines

On 6 November 2001, substrate samples for analysis of bulk chemical content and crystal structure of minerals were collected by hand from the surface of the stream substrate and wet-sieved through a 1 mm mesh sieve with stream water taken from the collection site. In some cases, material collected consisted entirely of fines that did not require sieving. A sample of sludge from the fish hatchery wastewater clarifier was collected on 9 November 2001. Fines (unsieved or sieved <1 mm) and sludge samples were collected in plastic centrifuge tubes and transported on ice to the Materials Research Laboratory (MRL) at Penn State University (PSU) on the same day that they were collected. The samples were dried at room temperature and crushed with a mortar and pestle to produce a powdered sample. Subsamples of the powdered material were submitted to the

Agricultural Analytical Services Laboratory at PSU for analysis of phosphorus content (see below). Additional subsamples were subjected to thermal gravimetric analysis (TGA) at the Materials Research Laboratory (MRL) at PSU (see below) to determine percent carbonate and percent organics.

X-ray diffraction (XRD) was used to assess the crystal structure of minerals in substrate samples. XRD analyses were conducted by Nichole Wonderling, MRL, PSU. The purpose of the XRD analyses was to assist in determining the source(s) of fines collected from Big Spring Creek. Samples of sludge from the fish hatchery effluent clarifier and samples of fines collected from BS0 were collected for comparison. A closer resemblance of the mineral crystal structure profile of fines collected from the stream to fines collected from BS0 or from the clarifier would indicate that the spring or the fish hatchery, respectively, was the more likely source of fines in the stream. A subsample of the powder was then placed in a cavity in a quartz zero-background sample holder. Samples were analyzed using a Scintag Pad V (Cupertino, CA) X-ray diffractometer, scanning in continuous mode from 10-70 degrees 2-theta at a rate of 2.0 deg/minute. Data analysis was performed using Scintag's DMSNT (version 1.37) powder diffraction software (Cupertino, CA).

Phosphorus content of fines

The Agricultural Analytical Services Laboratory, PSU, determined total phosphorus content in substrate samples collected from Big Spring Creek and Letort Spring Run on 6 and 9 November 2001. Air-dried and powdered samples were digested using U.S. EPA Method 3051 (U.S. EPA 1986a) and analyzed for total phosphorus content using U.S. EPA Method 6010B (U.S. EPA 1986b).

Organic and carbonate content of fines

Air-dried, powdered samples of fines from stream substrates were analyzed by thermal gravimetric analysis (TGA) to determine percent organics and percent carbonate. Dr. Raafat Malek, MRL, PSU, conducted the TGA analyses using the TGA 2050 thermogravimetric analyzer (TA Instruments, New Castle, DE). The TGA 2050 has a temperature range of ambient to 1000 °C. Balance noise is less than 0.1 microgram. Maximum sample capacity is 1.5 grams with a dynamic weight loss range of 0.1 microgram to 1.0 gram. The instrument features automated sample pan loading and un-loading capability, automated furnace movement control and post-run air-cooling. It uses a dual flow-meter for purge gas control inside the furnace and the balance compartments. The control software is Thermal Advantage (TA Instruments, New Castle, DE), which allows for automated step-wise isothermal heating with an output for percent weight loss per minute or micrograms loss per minute. The samples were run from room temperature up 1000 °C with a heating rate if 10 °C/min. The purge gas was argon. The data were analyzed using Universal Analysis (TA Instruments, New Castle, DE) software, which allows for the calculation of absolute weight loss and percent weight loss within certain temperature ranges.

Distribution of trout redds in Big Spring Creek

During fall 2001, sections of Big Spring Creek were surveyed to determine distribution of trout redds. Surveys were conducted on 30 October, 6 November, and 29 November. The 30 October survey began at the stone bridge on Spring Road, approximately 1 km upstream of Newville and ended at Big Spring, a distance of 4.15 km. Subsequent surveys began at the Nealy Road bridge and ended at Big Spring, a distance of 2.52 km. The reach downstream of the Nealy Road bridge was not surveyed in November because of the absence of potential spawning habitat. Redds were identified by the characteristic clearing of periphyton from the substrate along with a mound of gravel and a pit where a fish had buried eggs. Locations were noted where fish had cleaned the substrate but had not deposited eggs, i.e., periphyton was disturbed, but the characteristic mound and pit were absent. This is a common phenomenon in streams with embedded substrates. Locations of trout redds were mapped using Trout Unlimited Topo USA software (DeLorme, 2000). On 6 November 2001, depth and stream velocity (at 0.6 × depth) were recorded over some redds with a Marsh McBirney Model 2000 flowmeter. Substrate samples were collected from some redds (see above, **Texture of stream substrate**) to estimate the size frequency distribution of substrate particles. In January 2002, several redds were excavated to determine the stage of embryo development. On 27 February 2002, sixteen redds were excavated to determine whether embryos were present and the percentage of live embryos.

RESULTS AND DISCUSSION

Distribution of trout redds in Big Spring Creek

The November 6 survey revealed that the stream reach from the stone bridge to Nealy Road had good depth and cover for trout, but very little gravel. Redds (# 1-2) were initially encountered at the first bridge (private wooden bridge) upstream of the starting point (Table 2). Sixty-five total redds were observed among the three surveys. No redds were found near the BS2 sampling site at Nealy Rd., although there were numerous areas where fish had cleaned the substrate but had not constructed redds, presumably because they did not find the conditions suitable. The majority of redds (57/65) were located between BS1 (near the remnants of the old mill dam structure at the downstream end of the ditch) and a point approximately 400 m upstream of BS2. Four redds (#26, 26a, 26b, 26c) were located near the interstitial water sampling probe at BS1. Redds #28-30 were located near the BS1 sampling site just downstream of the remnants of the old mill dam structure. Only three redds (#31-33) occurred in the ditch; two were just upstream of the old mill dam structure, and one was 10 ft downstream of the hatchery outfall. Redd #34a (possible redd) was located directly in the flow of the hatchery outfall, and redd #34 was upstream of the hatchery outfall.

On 24 January 2002, several redds (#25-32) were excavated to determine stage of development of the eggs. Some live and dead eyed eggs were found. On 27 February 2002, the remaining redds from which substrate samples had been collected previously were excavated. The watercress had died back considerably since the last site visit in

January. This die-back appeared to have resulted in wider channels with reduced stream flow, shallower depths, and reduced velocities at redd locations. Redds at many locations were not distinct, i.e., the pit and downstream mound had changed to a more uniform bottom contour, presumably due to deposition of fine material in the pit. Others have noted loss of brown trout (Ottaway *et al.* 1981) and brook trout (Witzel and MacCrimmon 1983) redd structure over time during the egg incubation period. Redd structure is thought to improve infiltration of oxygenated surface water into redds (Kondolf 2000). However, Ottaway *et al*. (1981) noted that it is common for the first spate following redd construction to diminish brown trout redd structure prior to emergence of alevins and that loss of redd structure may be inconsequential to survival rate. Small fish, approximately 30 mm in length, were observed near redd #3. Additional fish of the same size were captured near redd #19 and determined to be trout. Based on their size, the fish probably had emerged one month earlier. In general, many of the excavated redds were determined by visual inspection to consist largely of sand or silt, whereas substrate samples previously collected from redds appeared to be comprised of more predominant gravels. At redd #12 and another site upstream, several egg masses were found; these contained eyed eggs, probably belonging to sculpins. Dead eggs were found in four redds (#12, #24, #25, #32); no live embryos or remains of eggs were found in nineteen redds.

Redd density at Big Spring Creek is in the low to moderate range at 27 redds per mile, as calculated from combined data collected in redd surveys conducted on 30 October 2001 and 29 November 2001. For comparison, redd density in Spring Creek, another Pennsylvania limestone stream, averages more than two-fold greater at 67 redds per mile, based on a 5-year average up to and including 2002. However, the redds in Big Spring Creek were dug by rainbow trout, brook trout, or brown trout, while redds in Spring Creek were dug by brown trout only.

Water velocity and depth

Based on criteria presented in Table 3, water velocity and depth in Big Spring Creek in November, prior to the watercress die-back, were adequate for brook trout and brown trout spawning. Since brook trout will spawn in lakes, surface water velocity is not required for survival of their embryos, but brook trout redds in lakes usually are located in areas of upwelling groundwater that replenish dissolved oxygen concentrations in the redds (Raleigh 1982). Some minimum surface water velocity would be required for brook trout embryos to incubate in streams without groundwater upwelling.

Table 2. Field notes from Big Spring Creek: redd survey, substrate sampling, and egg monitoring, 30 October 2001 and 6 and 29 November 2001. Redds marked with a number only were first observed on 30 November. A redd marked with a number and letter was observed the same area as the redd with the same number, but in subsequent surveys, with the date observed indicated under notes. PFBC= Pennsylvania Fish and Boat Commission. Depth and stream velocity were recorded at some redds on 6 November 2001. Redds #25 - 32 were excavated on 24 January 2002 to determine presence and number of eggs or larvae and stage of development. Sixteen redds were excavated on 27 February 2002. N= no eggs found, L= live egg, D= dead egg, E= eyed egg, S= egg shell (likely hatched), F= trout hovering near redd. Superscript numbers in parentheses indicate the redd sampled.

Redd #	Notes and description of location	Depth (m)	Velocity (m/s)	Eggs (01/24)	Eggs (02/27)
1	Immediately below wooden private bridge (first bridge upstream of starting point), 3 pits in road gravels.				
2	60 yd upstream of bridge. Several cleaned areas noted between bridge and redd.				
--	Nealy Rd. Numerous cleaned areas, but no redds.				
3	72 yd downstream of PFBC sign in parking lot. Parking lot is about 600 yd upstream of Nealy Rd. Redd was on right side of midchannel boulder just upstream of a V-rock deflector.	0.22	0.43		N
3a	Possible redd opposite side of same boulder. (11/29/2001)				*
4, 5	6 yd upstream of redd #3. Redds on either side of midchannel boulder.	0.20 (4)	0.55 (4)		N (4) * (5)
5a	Right side of channel 52 yd downstream from PFBC sign. (11/29/2001)				*
5b, 5c	Either side of old log directly across from large old tree in PFBC parking lot. (11/29/2001)				*
6	10 yd from PFBC sign, along cress bed.	0.35	0.64		N
7	13 yd from sign, midstream.				*
8	24 yd downstream of telephone pole 04903573 and 232 yd downstream from white barn on right.	0.28	0.63		N
8a	(11/06/2001)	0.26	0.60		N
9	166 yd downstream from wooden bridge leading to llama farm.	0.34	0.59		N
9a	183 yd downstream from wooden bridge leading to llama farm. (11/29/2001)				*
10	50 yd downstream from 30 mph sign, opposite white llama barn, midstream.				*
10a	Small redd 20 yd downstream of 30 mph sign. (11/29/2001)				*
11	50 yd downstream from wooden bridge, midstream, 5 yd from willow tree next to road.	0.32	0.65		N
11a	3.3 yd upstream from redd #11. (11/06/2001)				*
12	11 yd downstream from wooden bridge.	0.35	0.71		D, S [b]
13	Under wooden bridge, on right side of channel near pier.	0.26	0.43		N
13a	20 yd upstream from wooden bridge. (11/29/2001)				*

14,15,16	Midchannel opposite animal access, white barn, about 75 yd upstream of bridge leading to llama farm.	0.49 (14) 0.50 (16)	0.36 (14) 0.36 (16)		N (14,16) * (15)
14a	62 yd upstream from bridge to llama farm, across from pole with green electrical box. (11/29/2001)				*
16a	40 yd downstream of cement structure at lower end of PFBC parking lot. (11/29/2001)				*
16b	Between cement walls, on left side of channel. (11/29/2001)				*
16c	Mid-channel across from first square metal structure, lower side of bridge in PFBC lot. (11/29/2001)				*
16d	20 yd upstream from bridge at PFBC parking lot. (11/29/2001)				*
16e	32 yd upstream of PFBC sign. (11/29/2001)				*
16f	16 yd downstream of fish barrier, on right side of channel. (11/29/2001)				*
17	92 yd upstream of fish barrier, midstream.				*
17a	100 yd downstream of 30 mph sign, 100 yd upstream from fish barrier. (11/29/2001)				*
17b	16 yd downstream of boulder. (11/29/2001)				*
17c	Beside same boulder that is 16 yd down of flagged redd 18. (11/29/2001)				*
18	140 yd upstream from fish barrier, midstream, in coarse gravel.	0.46	0.22		N
19	30 yd upstream of redd #18, on left side, in large gravel.	0.22	0.64		N
19a	5 yd upstream of redd #19. (11/06/2001)				N
20,21,22	10 yd upstream of redd #19, 20 yd from 30 mph sign.				*
23	Right side directly opposite 30 mph sign.				*
23a	16 yd downstream of wooden post. (11/29/01)				*
23b	76 yd upstream of 30 mph sign. (11/29/01)				*
23c	12 yd upstream of redd #23b. (11/29/01)				*
24	118 yd upstream of 30 mph sign, nearly opposite drain pipe under road.	0.28	0.43		D [c]
24a	Across from black 653 mailbox. (11/29/01)				*
25	Midstream, 24 yd upstream and angled from red spring house.	0.18	0.45	N, F	D, S [d]
25a,b,c	Near red spring house. (11/29/01)			N	
26	3 yd below Spring Road Bridge at bottom of the ditch, on right side along bank.	0.18	0.51	N	
26a,b,c	(11/06/2001)			N	
27	5 yd upstream of middle pier of Spring Road Bridge.	0.18	0.46	N	
28,29,30	10 yd downstream of old dam, just upstream of v-rock deflector	0.14 (28) 0.20 (29) 0.23 (30)	0.43 (28) 0.41 (29) 0.38 (30)	N	
31	Immediately upstream of old mill dam. Two large brown trout on redd.			N	
32	4 yd upstream of redd #31 on right side.			D, E [a]	
33	3.3 yd below hatchery outfall, midstream.				
34	Brook trout on redd downstream of hatchery intake on far bank, at depth of approximately 3 ft				
34a	Possible redds directly in flow of hatchery outfall. (11/29/01)				

[a] Some dead eggs and some live eggs. Eggs were eyed, small, and did not appear to be ready to hatch.
[b] 27 dead eggs, 2 egg shells.
[c] One dead egg.
[d] Three dead eggs, one shell.
* Presence of redd was no longer evident.

Table 3. Spawning site conditions for brook trout and brown trout.

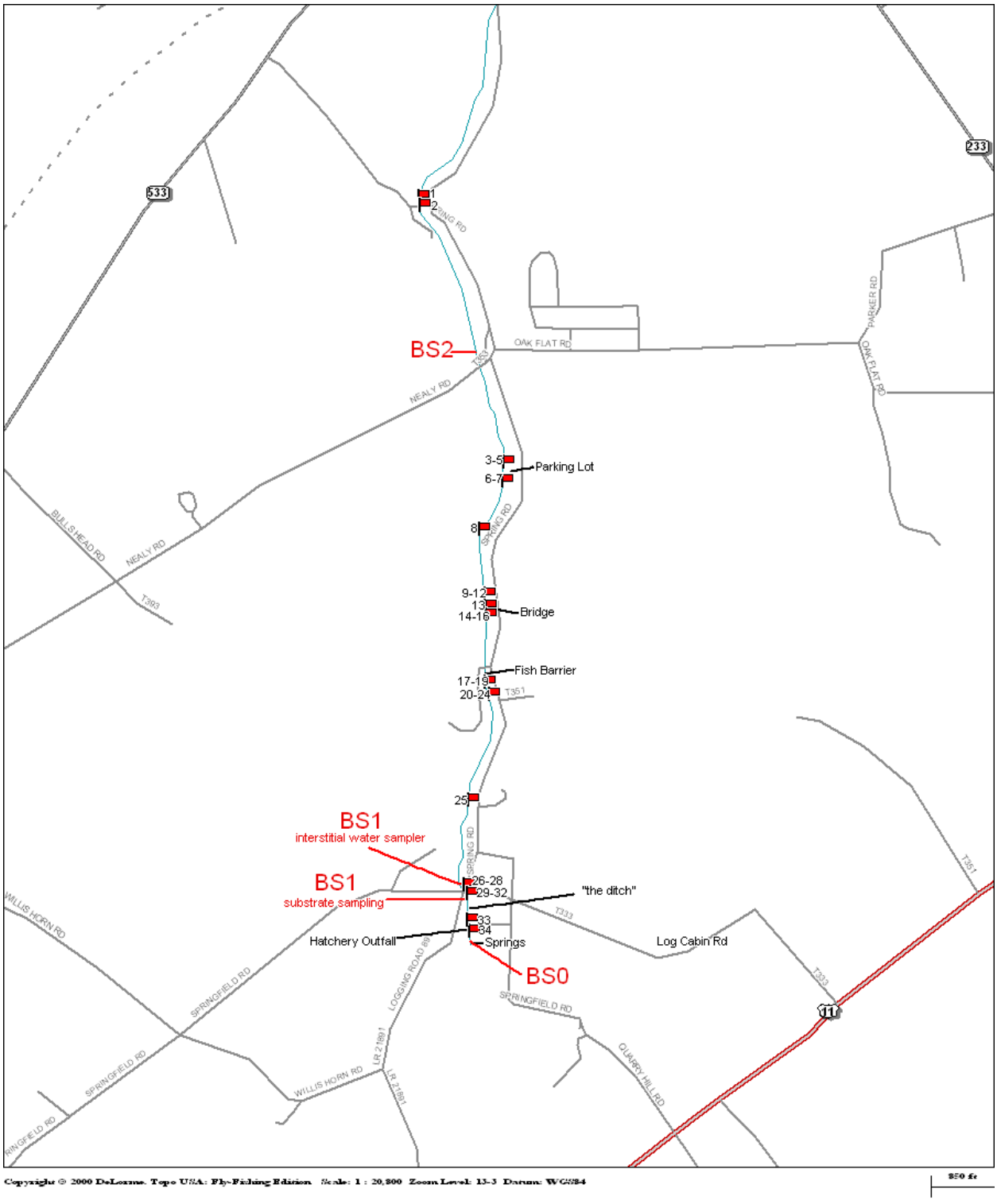
BROOK TROUT

Parameter	Normal range or criterion	Reference
velocity	11.2 –17.6 cm/s (0.112 – 0.176 m/s) (criteria)	Witzell and MacCrimmon 1983
	0.01 – 0.92 m/s (criterion)	Raleigh 1982
depth	means ranging from 13.4 – 24.9 cm (criteria) (0.134 – 0.249 m)	Witzell and MacCrimmon 1983
	mean depth 0.081 m (range 0.030 – 0.015 m)	Young *et al*. 1989
substrate size	mean substrate size criterion 5.7 mm	Witzell and MacCrimmon 1983

BROWN TROUT

Parameter	Normal range or criterion	Reference
velocity	30.8 – 46.5 cm/s (0.308 – 0.465 m/s) (criteria range)	Witzell and MacCrimmon 1983
	0.30 – 0.40 m/s	Ottaway *et al*. 1981
depth	criteria (means) ranging from 8.9 – 42.6 cm (0.089 – 0.426 m)	Witzell and MacCrimmon 1983
substrate size	mean substrate size criteria range 6.9 – 85.7 mm	Witzell and MacCrimmon 1983

Figure 3. Map of trout spawning locations in Big Spring Creek in fall and winter of 2001 – 2002.



Surface water quality and substrate interstitial water quality

Water quality data for Big Spring Creek and Letort Spring Run are reported in Tables 4 - 11 and in Figures 4 – 5. Because of the small number of water quality data points available for analysis, the following assessments are not based on results of statistical tests unless otherwise stated.

Temperature

According to Bjornn 1991, brook trout grow and survive best at 13 – 18 °C and typically spawn at 4 – 13 °C. The habitat suitability index model for brook trout by Raleigh (1982) assumes a temperature range of 0 – 24 °C and an optimal range of 11 – 16 °C for growth and survival of brook trout. Surface water temperatures were within 11 – 18 °C for all sites on Big Spring Creek and Letort Spring Run (no measurement in June) during sampling events from 28 June through 5 December. Surface water temperatures in January were less than 11 °C (minimum 7.2 °C for BS3) for all sites, but were still within the tolerance range for brook trout. The habitat suitability index model by Raleigh (1982) reports an optimum temperature range of 4.5 – 11.5 °C for brook trout egg incubation. Interstitial water temperatures were greater than the optimal temperature range for brook trout egg incubation prior to 24 January 2002 but were similar for Big Spring Creek and Letort Spring Run, a stream that supports a healthy reproducing population of brown trout.

Dissolved oxygen

Mean surface water DO concentrations measured during the incubation period for trout eggs (30 October 2001 to 24 January 2002) show an increase with distance from the spring source on both Big Spring Creek and Letort Spring Run (Figure 4). Embeck (2000) noted a similar increase in DO with distance from the hatchery discharge, but because DO was less below the discharge than at the spring source, he concluded that the hatchery effluent was producing a DO sag at the upstream sites. In contrast, DO concentrations measured at BS1 (just downstream of the ditch) in our study were similar to or slightly greater than DO concentrations measured near the spring. Mean surface water DO was greater near the spring source on the Letort than near the Big Spring. Although the sites on Letort Spring Run generally were sampled later in the day than those at Big Spring Creek, the difference in DO concentrations near the spring sources does not appear to be due to variation in time of day because the difference was still observed when sampling times were similar (Tables 10-11).

Surface water DO concentrations measured in “the ditch” on 26 October 1996 and at 0.6 mi. (0.97 km) and 1.5 mi. (2.4 km) from the spring on 29 June 1995 suggest that diurnal fluctuations in DO occur in Big Spring Creek (Black and Macri 1997). When such fluctuations occur, surface water measurements made during the daylight hours do not represent the worse case scenario, since DO levels would be expected to decline at night. On 27-28 June 2001, we measured DO in surface water and in substrate interstitial water at all sites in Big Spring Creek in the late afternoon or early evening and again near dawn

the following morning (Table 7). Surface water DO did not appear to differ between morning and afternoon/evening at BS1, but an increasing difference between morning and afternoon/evening DO levels was observed with increasing distance downstream.

Surface water DO levels in both Big Spring Creek and Letort Spring Run were adequate to support the health of adult trout. Optimum DO levels for adult brook trout appear to be ≥ 7 mg/L at temperatures less than 15 °C and ≥ 9 mg/L at temperatures ≥ 15 °C (Raleigh 1982). The U.S. EPA (1986c) coldwater minimum criterion for fish older than 30 days post hatch (dph) is 6.5 mg/L (30 day mean) with a 7-day mean minimum of 5.0 mg/L and a 1 day minimum of 4.0 mg/L. Pennsylvania water quality criteria are more stringent for waters falling under the Exceptional Value Waters (EV) and Cold Water Fishes (CWF) protected use designations. Big Spring Creek is listed as EV from the source to SR 3007 (T333) and as CWF from SR 3007 to the mouth (The Pennsylvania Code, Chapter 93). The Pennsylvania DO criterion for CWF is a minimum daily average of 6.0 mg/L and a minimum of 5.0 mg/L, while existing water quality must be maintained for the EV section (The Pennsylvania Code, Chapter 93). Assuming that our individual DO measurements are representative of mean DO for each site, DO concentrations in surface water in both streams (Figures 4, 5) were greater than the U.S. EPA coldwater minimum criterion for fish >30 dph and fell within the optimum range for brook trout.

Early life-stages (ELS) of fish are more sensitive to low DO than are older fish. Minimum intragravel DO levels for survival of salmonid embryos appear to vary to some degree with temperature but generally fall within the range of 2 - 8 mg/L (Kondolf 2000). According to Bjornn (1991),

> "Phillips and Campbell in 1961 concluded from field studies that intragravel concentrations of dissolved oxygen must average 8 milligrams per liter for high survival rates of embryos and alevins. Although concentrations of dissolved oxygen required for successful incubation depend on the species of fish and developmental stage, concentrations at or near saturation, with temporary reductions no lower than 5 milligrams per liter, will probably allow high survival of salmonids in most cases."

The U.S. EPA (1986c) ambient DO criterion to protect ELS of fish (embryonic, larval, and juvenile forms up to 30 dph) is a surface water criterion designed to achieve a corresponding intergravel DO criterion, assuming a difference of 3 mg/L between surface water and intergravel water. The coldwater criterion for protection of ELS is a 7-day mean of 9.5 mg/L with a 1 day minimum of 8.0 mg/L (U.S. EPA 1986c). This surface water criterion was designed to achieve a 7-day mean DO of 6.5 mg/L in intergravel water (based on the expectation of slight impairment at a mean threshold DO concentration of 6 mg/L), with a 1 day minimum of 5 mg/L in intergravel water (U.S. EPA 1986c). Since we measured interstitial water DO directly rather than estimating it from surface water DO concentration, the surface water criterion for protection of ELS probably is less critical, particularly since the assumption of a 3 mg/L difference would have overestimated interstitial DO at BS2 and BS3 (See Table 12). During the trout incubation period, the only site with interstitial DO concentrations less than the U.S. EPA coldwater minimum criterion for protection of ELS was BS3 (6.44 mg/L on 5 December 2001 and 6.16 mg/L on 24 January 2002); these concentrations are very close to the

threshold for slight impairment of ELS. DO concentration in interstitial water at BS2 was 6.51 mg/L on 30 October 2001. Even if the measurements are representative of average DO concentrations, the safety margin for hypoxia in interstitial water at BS3 is small. If diurnal fluctuations in DO concentrations occur in interstitial water as they do in surface water at these sites, the interstitial DO might be expected to drop below the U.S. EPA ambient water quality criterion at BS2 and BS3 at night during the trout incubation period. Measurements of surface water DO were not less than 8 mg/L (the 1 day minimum) for any site sampled. Surface water DO was less than the 7-day mean criterion of 9.5 mg/L during the trout incubation period at BS0 on 30 October 2001 (8.74 mg/L), 5 December 2001 (8.74 mg/L) and 24 January 2002 (9.20 mg/L) and at BS1 on 5 December 2001 (9.18 mg/L), but the corresponding interstitial water DO concentrations exceeded the criterion for interstitial water on those same days.

Interstitial DO levels less than 5 mg/L have been measured previously at locations near BS2 in Big Spring Creek in April 2000 (Embeck 2000). In the winter of 1995-96, Black and Macri (1997) measured interstitial DO at 4 ppm within an artificial redd 50 feet downstream of the Spring Road Bridge (Black and Macri 1997). In January 1996, interstitial DO between 4 and 5 ppm was measured in or next to natural redds 0.25 mi. (400 m) and 0.6 mi. (966 m) downstream of Spring Road Bridge (between the Spring Road Bridge and the fish barrier). Two interstitial water DO measurements were taken outside the trout incubation period, on 6 June 2001 (3.00 mg/L at BS2 and 5.00 mg/L at BS3); measurements were not completed at BS0 and BS1 due to DO meter failure. Trout in Big Spring Creek would not be exposed directly to substrate interstitial water during the summer months.

Although the interstitial water samples taken in June were collected using a syringe rather than a sampling probe, the differences between surface water DO and interstitial water DO were consistent with differences measured in other months, indicating that the measurements made in June are reliable. DO concentration was greater in interstitial water samples than in surface water samples at BS0 on 5 December 2001 and on 24 January 2002. Groundwater upwelling, which was observed along with rising air bubbles near the interstitial water sampling probe at BS0 on 30 October 2001, might explain this unusual finding.

Table 12 lists the differences between DO in surface water and DO in substrate interstitial water, or the DO differential, for each site. The DO differential at Embeck's Location 1 (2.2 mg/L) was greater than the mean differential at our comparable BS1 site, while the differential at his Location 3 (3.5 mg/L) was less than the mean differential at our analogous site BS2. The DO differential at Embeck's Letort Spring Run location (1.3 mg/L) is slightly less than the average differential at our comparable LE1 site. The reason for the dramatic increase in the DO differentials at both Letort Spring Run sites on 24 January 2002 is not known. Similar surface water DO and substrate interstitial water DO concentrations between sites BS0 (upstream of the hatchery discharge) and BS1 (just downstream of the hatchery discharge) in Big Spring Creek during the trout incubation period suggest that the hatchery effluent was not producing a negative impact on that parameter during the period of embryo development and hatching in 2001/2002.

However, it is important to note that this conclusion is based on only a the few surface water DO measurements made in this study and does not consider past conditions associated with hatchery effluent.

Oxidation-reduction potential

Oxidation-reduction potential (ORP), or redox potential (Eh), is an indicator of the relative degree of carbon enrichment in sediments (Pearson and Black 2001). Positive ORP values are associated with aerobic conditions, while negative values are associated with anaerobic microbial processes (Pearson and Black 2001). ORP profiles measured in sediments to a depth of 10-15 cm have been used to assess the degree to which waste from fish cages used for marine aquaculture enrich the underlying marine sediments. Reduced ORP values, including negative values, were measured in sediment cores collected from a highly enriched area adjacent to a fish cage relative to reference locations some distance from the cage (Pearson and Black 2001). ORP was measured in surface water and in interstitial water in Big Spring Creek and in Letort Spring Run as an indicator of organic enrichment. On 28 June, ORP values measured in surface water and in interstitial water did not demonstrate any clear trends that would indicate a point source of organic enrichment (Tables 5 and 6). ORP in surface water was slightly lower in the afternoon or evening than in the early morning at each site in Big Spring Creek (Table 7). The greatest ORP values were measured in surface water at BS2. Surface water ORP values measured at BS0 and BS1 were similar. Interstitial water ORP was less at BS2 than at any of the other sites, but no comparison could be made to BS0 because it was not possible to collect an interstitial water sample with the apparatus available on that day. On 3 October, a gradient of decreasing ORP values was observed from BS0 downstream to BS3, but the least ORP value observed in Big Spring Creek was similar to values observed in Letort Spring Run (Table 8). ORP values seem to decrease with increasing time of day when samples were collected, so timing of sample collection cannot be discounted as a factor. On 30 October and 1 November, no interesting trend in surface water ORP values was observed (Table 9). However, interstitial water ORP was less at BS1 than at any other site in Big Spring Creek or Letort Spring Run, and this finding did not appear to have been influenced by sample collection time. On 5 December, ORP values in surface water and in interstitial water were less at BS1 and BS2 than at other sites in Big Spring Creek and were less than those at LE1 (Table 10). However, interstitial water ORP was substantially less at LE0 than at any other site in Big Spring Creek or Letort Spring Run. On 24 January, ORP values observed for BS1 (surface water and interstitial water) were comparable to those observed for sites in Letort Spring Run and greater than those measured at all other sites in Big Spring Creek (Table 11).

From 28 June 2001 through 24 January 2002, all ORP values were positive for both surface water and interstitial water (at least to a depth of 13 cm) at all sites studied, indicating that aerobic conditions prevailed in both Big Spring Creek and Letort Spring Run. Measured dissolved oxygen concentrations did not appear to vary with ORP.
Although ORP appeared to be reduced at BS1 in October or November and at BS1 and BS2 in December, ORP was elevated at BS1 in comparison with other sites in late

January, and there was no obvious trend in ORP in June. However, fish production at the hatchery was halted on 5 November and had been scaled down prior to that date, so it is impossible to know how the hatchery effluent might have affected ORP in previous years when trout production was more intense.

Un-ionized ammonia

Ammonia, particularly that in the un-ionized form (NH_3), is toxic to fish at all life stages. Exposure to concentrations of NH_3 as low as 0.027 mg/L resulted in 71.1% mortality of rainbow trout from egg through fry stage when the exposure began within 24 hr of fertilization; lesser concentrations were not tested (Solbé and Shurben 1989). It is generally accepted among fish culturists that NH_3 concentrations should not exceed 0.0125 mg/L to protect the health of salmonid fishes (Meade 1985). Growth rates of rainbow trout are reduced and damage to liver, kidney, and gill tissue may occur when NH_3 levels exceed 0.0125 – 0.025 mg/L (U.S. EPA 2002). Based on these previous studies, it will be assumed that un-ionized ammonia concentrations less than 0.0125 mg/L are safe for salmonids. In addition, the U.S. EPA has established pH-dependent Criterion Maximum Concentration (salmonids present, acute criterion) and temperature- and pH-dependent Criterion Continuous Concentration (fish early life-stages present, chronic criterion) for total ammonia (U.S. EPA 1999).

All total ammonia concentrations measured were less than the U.S. EPA water quality criteria for protection of early life-stages of salmonids in coldwater systems (See Appendix A) (U.S. EPA 1999). Total ammonia (NH_3-N) concentrations were measured twice during the egg incubation period, on 30 October 2001 or 1 November 2001 and on 5 December 2001. NH_3-N was low throughout the egg incubation period (Tables 9 - 11), and was detected above the method detection limit (MDL) of 0.006 mg/L only on December 5 (0.022 mg/L in BS2 surface water, 0.032 mg/L in BS3 surface water, and 0.007 mg/L in LE1 surface water). NH_3 would represent only a small percentage (< 1%) of the total ammonia concentrations at the observed pH and temperature ranges, so that NH_3 was sufficiently low throughout the egg incubation period to protect the health of salmonids. However, during incubation, the developing trout would excrete ammonia in addition to that which was measured in the substrates in the absence of eggs or alevins, and decomposition of dead eggs or alevins would contribute additional ammonia (Tappel and Bjornn 1983).

Outside the egg incubation period, ammonia in surface water and substrate interstitial water was measured on 6 June 2001 and on 30 October 2001 (Tables 5, 8). Un-ionized ammonia concentration reached a level exceeding 0.0125 mg/L in only one sample (0.0248 mg/L in BS2 substrate interstitial water on 6 June). Salmonid fishes that spawn in the fall would not have been exposed directly to substrate interstitial water during this time, and invertebrates, upon which the trout depend for food, generally are more tolerant of ammonia than are fish (U.S. EPA 1986c).

Total phosphorus

Total phosphorus concentrations in surface water were measured at all Big Spring Creek sampling sites on 28 June 2001, and at all sampling sites on Big Spring Creek and Letort Spring Run on 3 October 2001 (Tables 5 and 8, summarized in Table 4). In the absence of data from appropriate reference streams, the U.S. EPA recommends adoption of a criterion of 10.00 µg total P/L for rivers and streams in Aggregate Ecoregion XI, which includes Pennsylvania (U.S. EPA 2000a). All samples collected from Big Spring Creek and Letort Spring Run exceeded this value by more than four-fold. Since Letort Spring Run was identified as a reference stream for the purposes of this study, 44 µg P/L (average of values at LE0 and LE1 on 3 October 2001) might be a more appropriate number for comparison to total P measured in Big Spring Creek. The reference value of 44 µg P/L was exceeded at BS0 and BS1 in June and at BS0, BS1, and BS2 in October. It is interesting to note that two of the three greatest values measured were in samples collected at BS0, upstream of the hatchery effluent discharge. This might indicate that the Big Spring itself is a significant source of phosphorus in Big Spring Creek. A previous report asserts that the concentration of phosphorus at the spring is typically 0.02 to 0.03 mg/L (Embeck 2000); however, our measurements of total P near the spring source (BS0) were more than two-fold greater. In June 2001, total P in surface water decreased with increasing distance downstream. In October 2001, total P in surface water at BS0 was greater than that at BS1 and BS3, but BS2 had the greatest concentration of total P for all sites. This suggests that another source of phosphorus input occurs between BS1 and BS2. On June 28, interstitial water contained a much greater concentration of total P than did surface water at BS1, BS2, and BS3 (BS0 not measured), and total P was elevated in interstitial water at BS1 and BS2 relative to BS3. This trend suggests an influence of the hatchery discharge but the lack of a data point for BS0 limits the interpretation.

Table 4. Total phosphorus (P) (µg /L) measured in surface water at Big Spring Creek and Letort Spring Run in 2001.

	Total P (µg/L)	
Site	06/28	10/03
BS0	68	65
BS1	58	48
BS2	43	75
BS3	36	44
LE0	--	44
LE1	--	43

Table 5. Water quality data collected for surface water and substrate interstitial water from Big Spring Creek on 28 June 2001. Interstitial water samples were collected with a sampling needle.

Water Quality Parameter	Site							
	BS0 surface	BS0 interstitial[a]	BS1[d] surface	BS1[d] interstitial	BS2 surface	BS2 interstitial	BS3 surface	BS3 interstitial
sample collection time	6:08 AM		6:22 AM	noon	6:32 AM	10:30AM	6:46 AM	7:05 AM
temperature (°C)	11.1		11.3	12.3	12.0	19.9	15.1	17.7
pH[g]	7.6		7.6	7.6	7.7	7.5	7.8	7.6
dissolved oxygen (mg/L)[f]	10.4		10.4	X	8.2	3.0	9.1	5.0
ORP (redox potential, mV)	233		226	199	247	175	215	221
calcium (mg/L)	70.23		69.22	61.34	79.84	66.58	73.81	63.01
magnesium (mg/L)	10.08		12.00	10.02	13.76	10.10	11.60	11.46
total hardness (mg $CaCO_3$/L) (calc.)	217		222	194	256	208	232	205
total ammonia (mg N/L, NH_3-N)	0.011		0.114	0.053	0.026	1.661	0.015	< 0.006
un-ionized ammonia (mg/L)[b]	< 0.001		0.00113	< 0.001	< 0.001	0.0248	< 0.001	< 0.001
nitrite (mg N/L, NO_2-N)	0.026		0.017	0.024	0.041	0.045	0.056	0.073
total phosphorus (mg P/L)	0.068		0.058	1.727	0.043	1.73	0.036	1.002
alkalinity (mg $CaCO_3$/L)	164.9		168.7	293.7	156.6	507.1	195.9	197.4
TOC (mg C/L)	0.612		0.907	1.57	1.05	1.61	1.16	1.12
DOC (mg C/L)	0.538		0.686	0.799	1.36	1.45	1.00	0.906
POC (mg C/L)	0.074		0.221	0.771	-0.31[e]	0.16	0.16	0.214
BOD_5 (mg/L)[c]	4.79		3.79		15.9		20.4	

TOC= total organic carbon, measured. DOC= dissolved organic carbon, measured. POC= particulate organic carbon, estimated by subtraction, POC= TOC - DOC.

[a] No interstitial water sample was collected at BS0 because the substrate was rock and could not be penetrated by the sampling needle.

[b] Un-ionized ammonia (NH_3) concentrations calculated by method described by Thurston *et al*. 1979. Values were not corrected for salinity or TDS.

[c] BOD_5 was not measured in interstitial water because the volume that could be obtained in a single sample was not sufficient.

[d] Sample labeled BS1 was collected just downstream of old mill dam, upstream of bridge on Log Cabin Road.

[e] Reported value for TOC is less than that for DOC, resulting in a negative number for POC by subtraction. Sample was re-analyzed with similar results.

[f] Dissolved oxygen was measured with YSI Model 58.

[g] Measured with Oakton pHTestr 3.

Table 6. Water quality data collected from Big Spring Creek on 28 June 2001 for comparison of surface water and substrate interstitial water quality.

	Water Quality Parameter				
Site	collection time	temp. (°C)	pH	DO (mg/L)	ORP (mV)
BS0-surface	6:08 AM	11.1	7.6	10.4	233
BS0-interstitial	1:02 PM	10.9	7.4	X	208
BS1-surface	6:22 AM	11.3	7.6	10.4	226
BS1-interstitial	noon	12.3	7.6	X	199
BS2-surface	6:32 AM	12.0	7.7	8.2	247
BS2-interstitial	10:30 AM	19.9	7.5	3.0	175
BS3-surface	6:46 AM	15.1	7.8	9.1	215
BS3-interstitial	7:05 AM	17.7	7.6	5.0	221

temp.= temperature, DO= dissolved oxygen, ORP= oxidation-reduction potential (redox potential)

Interstitial water sample measurements should be viewed with skepticism because water samples were collected with a syringe.

X= no measurement made due to DO meter failure

Table 7. Surface water quality data collected on 27 and 28 June 2001 to compare morning and evening measurements.

Site	Water Quality Parameter					
	collection date	collection time	temp. (°C)	pH	DO (mg/L)	ORP (mV)
			Afternoon			
BS0	06/28	1:02 PM	10.9	7.4	X	208
BS1	06/27	6:54 PM	12.4	7.7	10.3	206
BS2	06/27	5:55 PM	17.2	7.9	9.0	226
BS3	06/27	7:43 PM	19.4	8.2	11.2	195
			Early Morning			
BS0	06/28	6:08 AM	11.1	7.6	10.4	233
BS1	06/28	6:22 AM	11.3	7.6	10.4	226
BS2	06/28	6:32 AM	12.0	7.7	8.2	247
BS3	06/28	6:46 AM	15.1	7.8	9.1	215

temp.= temperature, DO= dissolved oxygen, ORP= oxidation-reduction potential (redox potential)
X= no measurement made due to DO meter failure

Table 8. Surface water quality data collected from Big Spring Creek and Letort Spring Run on 3 October 2001.

Water Quality Parameter	Site					
	BS0	BS1[a]	BS2	BS3	LE0	LE1
sample collection time	12:15 PM	1:08 PM	2:29 PM	3:04 PM	4:19 PM	5:00 PM
temperature (°C)	11.0	12.1	15.5	17.1	11.3	13.1
pH	7.00	7.32	7.77	8.36	7.30	7.72
dissolved oxygen (mg/L)[c]	9.05	9.72	10.44	13.93	9.70	11.25
ORP (redox potential, mV)	282	269	252	171	187	152
calcium (mg/L)	70.55	70.06	70.26	73.9	88.70	93.72
magnesium (mg/L)	8.610	10.46	8.579	9.905	13.49	13.27
total hardness (mg $CaCO_3$/L)(calc.)	212	218	211	225	277	289
total ammonia (mg N/L, NH_3-N)	< 0.006	0.093	< 0.006	< 0.006	< 0.006	< 0.006
un-ionized ammonia (mg/L)[b]		5.16E-04				
nitrite (mg N/L, NO_2-N)	<0.013	<0.013	0.032	0.037	<0.013	<0.013
total phosphorus (mg P/L)	0.065	0.048	0.075	0.044	0.044	0.043
alkalinity (mg $CaCO_3$/L)	163.6	168.1	170.2	169.3	230.3	226.3
TOC (mg C/L)	0.380	0.711	0.964	1.142	0.541	0.706
DOC (mg C/L)	0.331	0.496	0.779	0.897	0.455	0.677
POC (mg C/L)	0.049	0.215	0.185	0.245	0.086	0.029

TOC= total organic carbon, measured. DOC= dissolved organic carbon, measured. POC= particulate organic carbon, estimated by subtraction, POC= TOC – DOC.

[a] Sample labeled BS1 was collected just downstream of old mill dam, upstream of bridge on Log Cabin Road.

[b] Un-ionized ammonia (NH_3) concentrations calculated by method described by Thurston *et al*. 1979. Values were not corrected for salinity or TDS. For samples with ammonia concentrations < MDL of 0.006 mg/L, un-ionized ammonia concentrations were not calculated because levels are too low to be toxicologically significant.

[c] Dissolved oxygen was measured with YSI Model 58.

Table 9. Surface water quality and substrate interstitial water quality data collected from Big Spring Creek and Letort Spring Run on 30 October and 1 November 2001.

Water Quality Parameter	Site									
	BS0 surface	BS0 interstitial	BS1 surface	BS1 interstitial	BS2 surface	BS2 interstitial	BS3 surface	BS3 interstitial	LE0 surface	LE0 interstitial
sample collection date	10/30	10/30	10/30	10/30	11/01	11/01	11/01	11/01	11/01	11/01
sample collection time	10:54 AM	noon	12:28 PM	1:58 PM	11:57 AM	12:30 PM	2:35 PM	2:57 PM	3:42 PM	3:50 PM
temperature (°C)	11.1	13.5	11.8	13.1	11.6	14.9	12.1	13.9	11.9	13.5
pH	7.51	7.84	7.67	7.60	8.00	7.90	8.10	7.80	7.60	7.90
dissolved oxygen (mg/L)[a]	8.74	8.11	9.82	8.54	11.06	6.51	12.10	8.70	10.51	9.10
ORP (redox potential, mV)	244	227	256	148	247	247	222	216	231	222
total ammonia (mg N/L, NH_3-N)[b]	< 0.006	< 0.006	< 0.006	< 0.006	< 0.006	< 0.006	< 0.006	< 0.006	< 0.006	< 0.006

[a] Dissolved oxygen values represent the average of measurements from two different dissolved oxygen meters (YSI Model 95 and YSI Model 58).
[b] Total ammonia levels were so low that it was not necessary to calculate un-ionized ammonia concentrations.

Table 10. Surface water quality and substrate interstitial water quality data collected from Big Spring Creek and Letort Spring Run on 5 December 2001.

Water Quality Parameter	Site											
	BS0 surface	BS0 interstitial	BS1 surface	BS1 interstitial	BS2 surface	BS2 interstitial	BS3 surface	BS3 interstitial	LE0 surface	LE0 interstitial	LE1 surface	LE1 interstitial
sample collection time	1:45 PM	2:05 PM	12:51 PM	1:23 PM	11:30 AM	12:10 PM	2:24 PM	2:33 PM	3:02 PM	3:06 PM	3:28 PM	3:33 PM
temperature (°C)	11.0	11.8	11.2	13.5	11.9	14.1	11.7	13.1	11.7	12.4	11.9	12.5
pH	6.98	7.08	7.04	7.16	7.93	7.36	7.60	7.26	7.42	7.40	7.44	7.47
dissolved oxygen (mg/L)[a]	8.74	8.87	9.18	8.51	10.93	7.72	11.30	6.44	10.41	9.59	10.37	9.92
ORP (redox potential, mV)	243	241	182	201	177	188	219	244	244	115	202	227
total ammonia (mg N/L, NH_3-N)[b]	< 0.006	< 0.006	< 0.006	< 0.006	0.022	< 0.006	0.032	< 0.006	< 0.006	< 0.006	0.007	< 0.006

[a] Dissolved oxygen values represent the average of measurements from two different dissolved oxygen meters (YSI Model 95 and YSI Model 58).
[b] Total ammonia levels were so low that it was not necessary to calculate un-ionized ammonia concentrations.

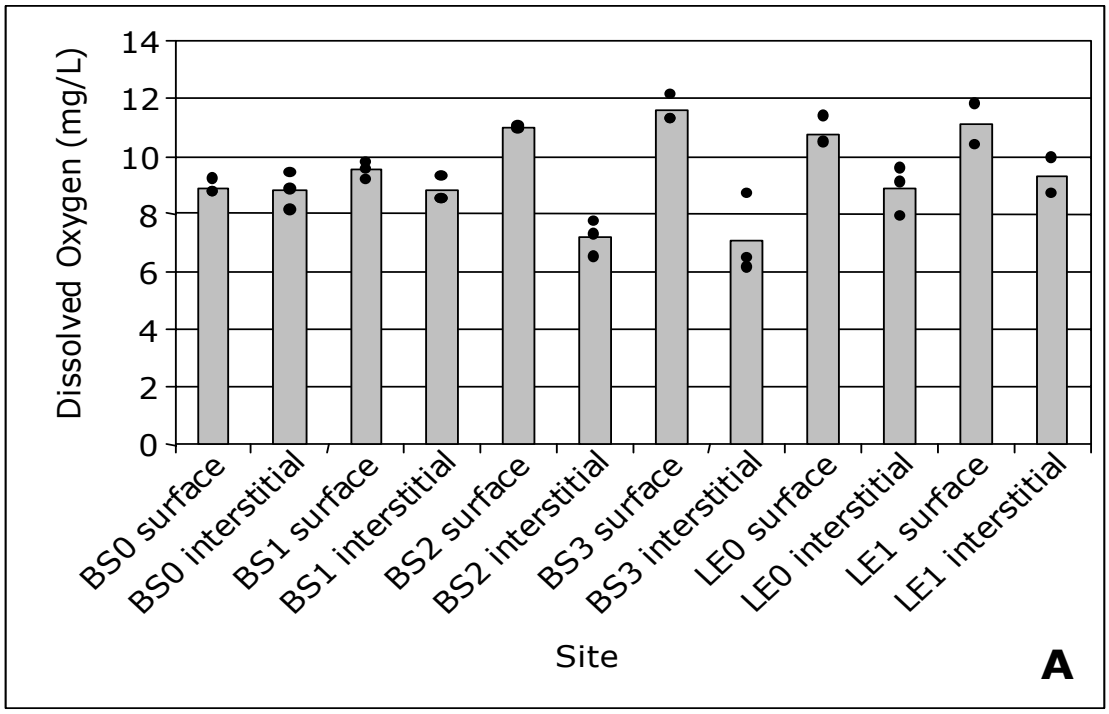
Table 11. Surface water quality and substrate interstitial water quality data collected from Big Spring Creek and Letort Spring Run on 24 January 2002.

Water Quality Parameter[a]	Site											
	BS0 surface	BS0 interstitial	BS1 surface	BS1 interstitial	BS2 surface	BS2 interstitial	BS3 surface	BS3 interstitial	LE0 surface	LE0 interstitial	LE1 surface	LE1 interstitial
sample collection time	11:25 AM	11:25 AM	1:00 PM	1:00 PM	11:00 AM	11:00 AM	10:15 AM	10:15 AM	noon	noon	12:24 PM	12:24 PM
temperature (°C)	10.9	10.3	10.8	10.7	9.5	9.0	7.2	7.9	10.5	10.1	10.0	9.6
pH	7.54	7.60	7.68	7.67	8.03	7.68	7.97	7.65	7.68	7.67	7.77	7.72
dissolved oxygen (mg/L)[b]	9.20	9.41	9.52	9.32	10.99	7.25	11.30	6.16	11.38	7.90	11.80	8.72
ORP (redox potential, mV)	36	38	54	48	37	31	20	36	53	49	44	52

[a] Ammonia levels were not measured because levels in past measurements were low, and temperature is low as well.

[b] Dissolved oxygen was measured with YSI Model 58.

Figure 4. Mean dissolved oxygen (DO) concentrations (mg/L) in surface water and substrate interstitial water from Big Spring Creek and Letort Spring Run. Shaded bars represent means. Solid circles represent individual data points. Figure 4.A.: Data (n=3 per site) collected during the egg incubation period for trout (10/30/2001, 12/05/2001, and 01/24/2002). Figure 4.B.: Data collected prior to egg incubation period for trout (06/27/2001, 06/28/2001, and 10/03/2001); sample sizes vary.



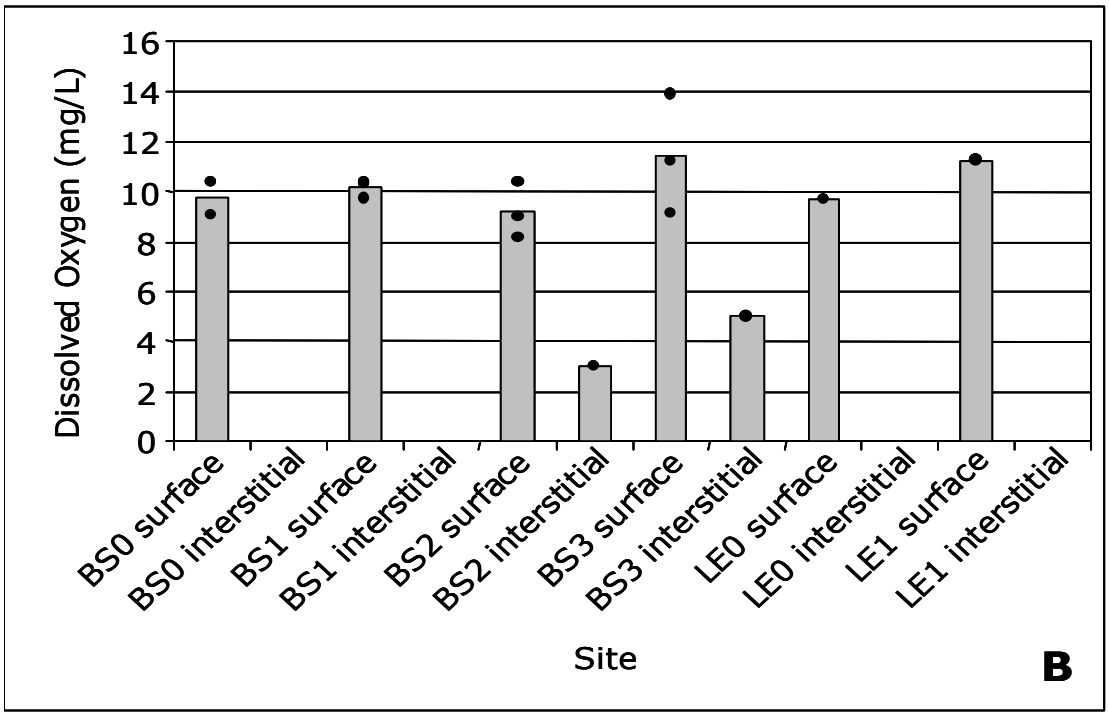
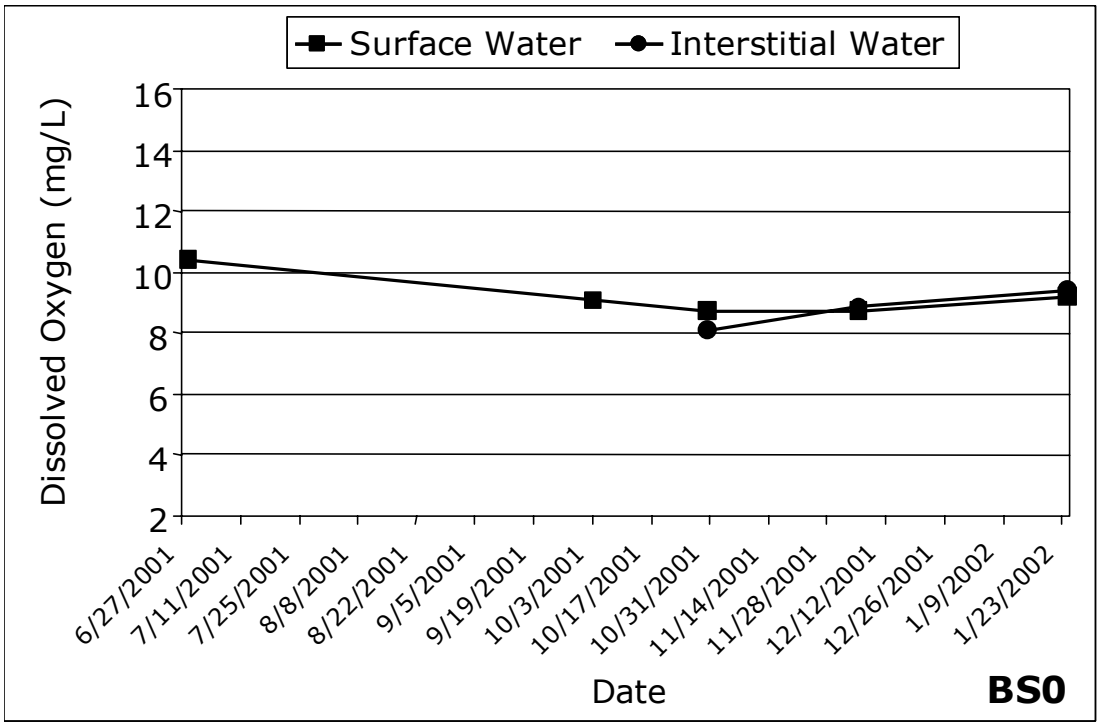
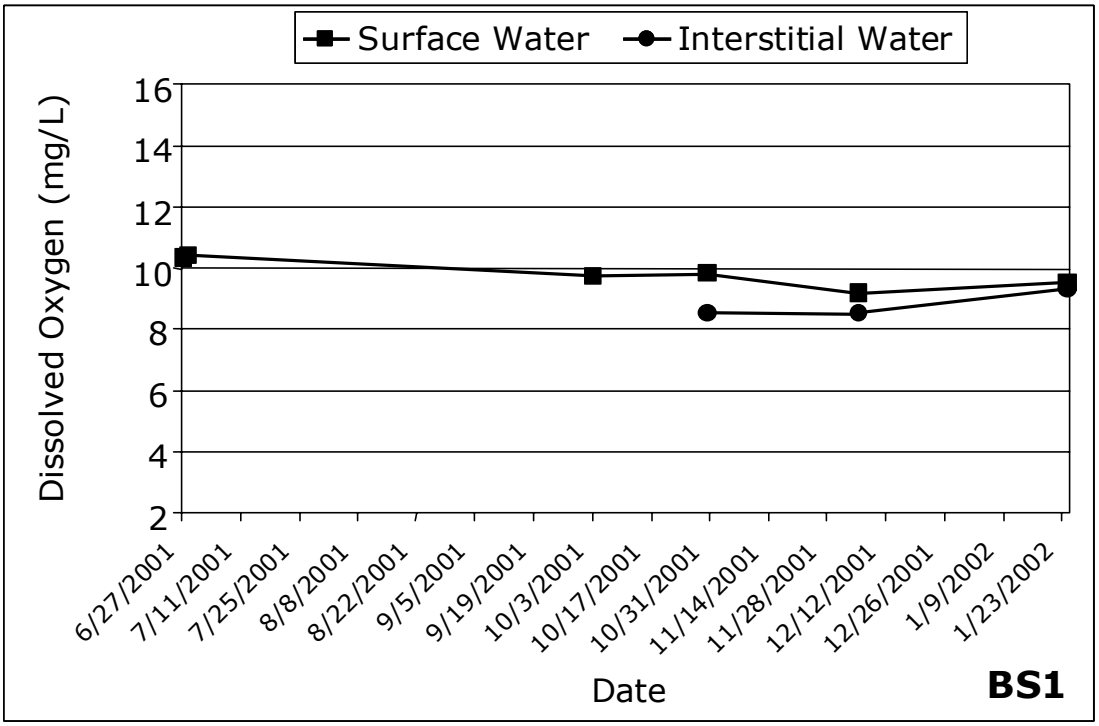
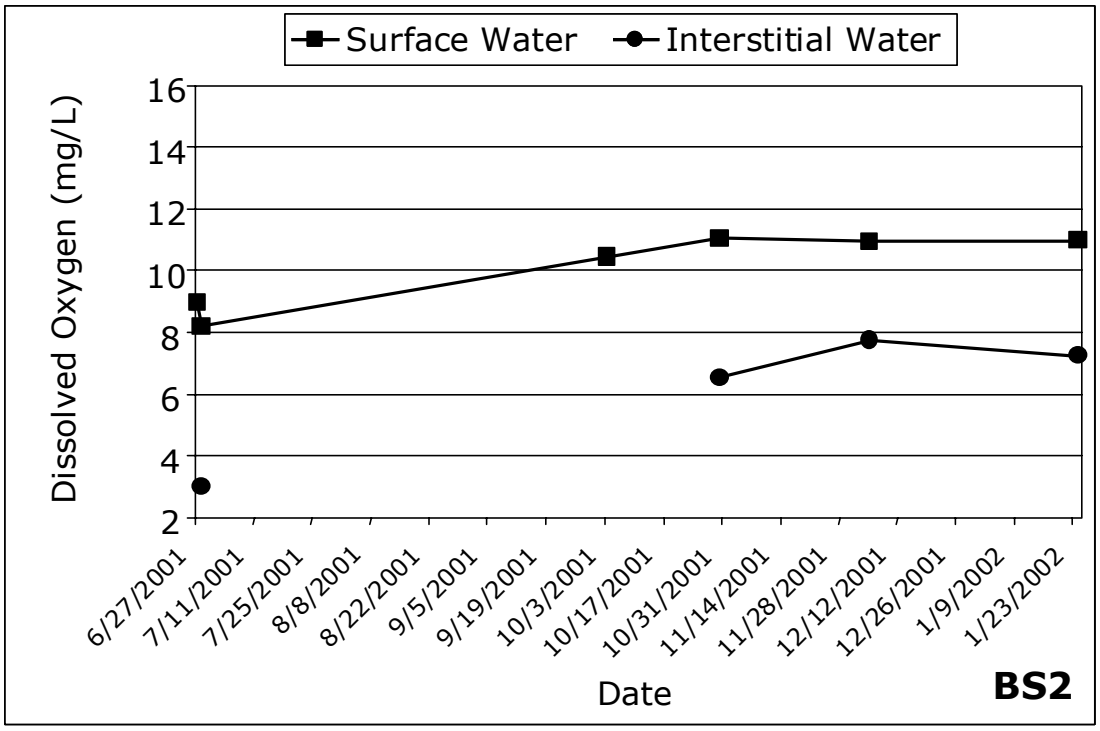


Figure 5. Dissolved oxygen (DO) concentrations in surface water and interstitial water at Big Spring Creek and Letort Spring Run, plotted over time within each site. DO measurements were made on the following dates: 06/27/2001, 06/28/2001, 10/03/2001, 10/30/2001, 12/05/2001, 01/24/2002.






Surface Water
Interstitial Water
Dissolved Oxygen (mg/L)
16
14
12
10
8
6
4
2
6/27/2001
7/11/2001
7/25/2001
8/8/2001
8/22/2001
9/5/2001
9/19/2001
10/3/2001
10/17/2001
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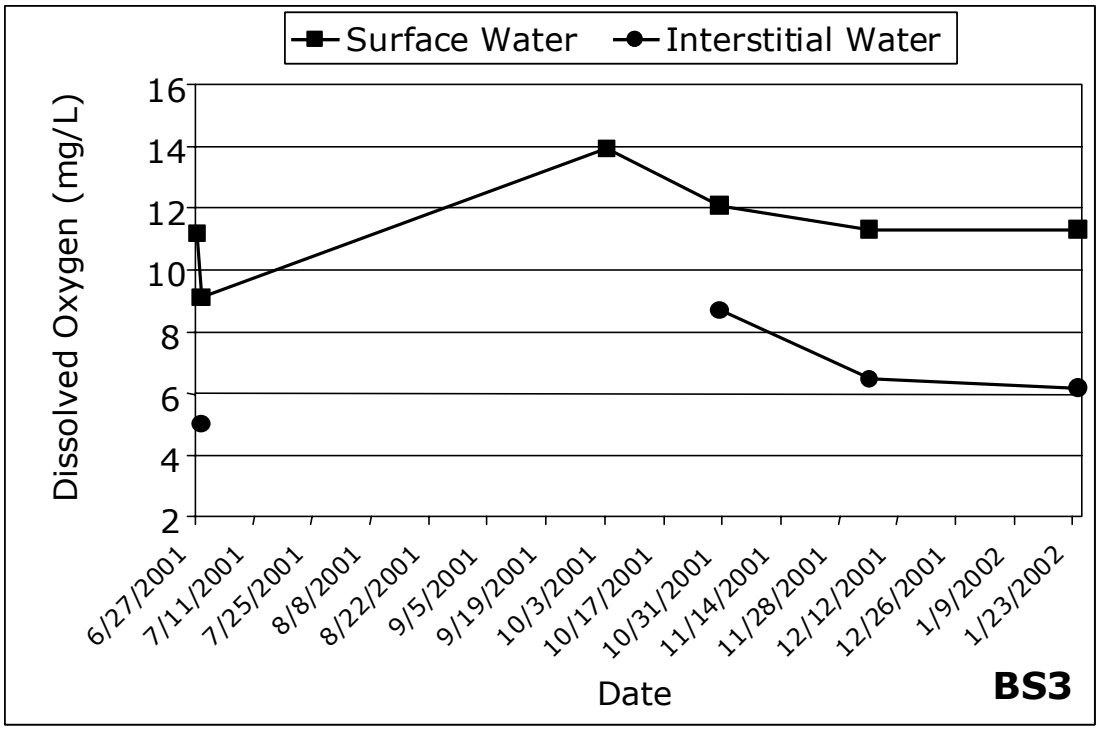
11/14/2001
11/28/2001
12/12/2001
12/26/2001
1/9/2002
1/23/2002
Date
BS2


Surface Water
Interstitial Water
Dissolved Oxygen (mg/L)
16
14
12
10
8
6
4
2
6/27/2001
7/11/2001
7/25/2001
8/8/2001
8/22/2001
9/5/2001
9/19/2001
10/3/2001
10/17/2001
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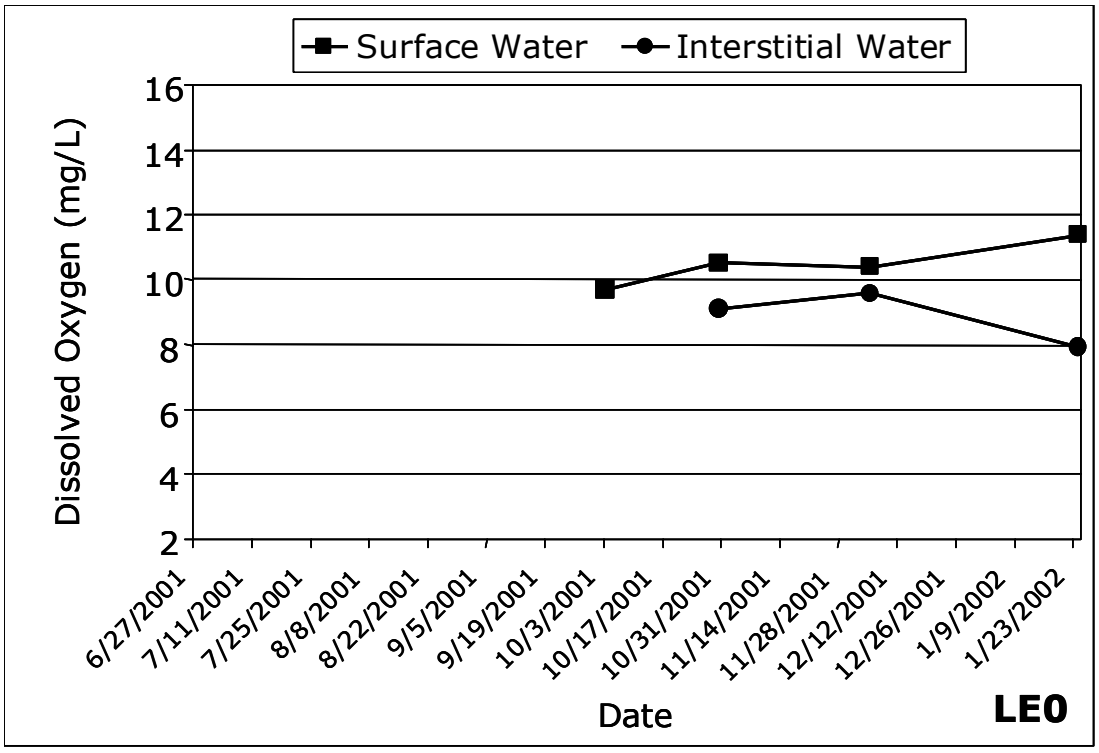
12/26/2001
1/9/2002
1/23/2002
Date
BS3


Surface Water
Interstitial Water
Dissolved Oxygen (mg/L)
16
14
12
10
8
6
4
2
6/27/2001
7/11/2001
7/25/2001
8/8/2001
8/22/2001
9/5/2001
9/19/2001
10/3/2001
10/17/2001
10/31/2001
11/14/2001
11/28/2001
12/12/2001
12/26/2001
1/9/2002
1/23/2002
Date
LE0

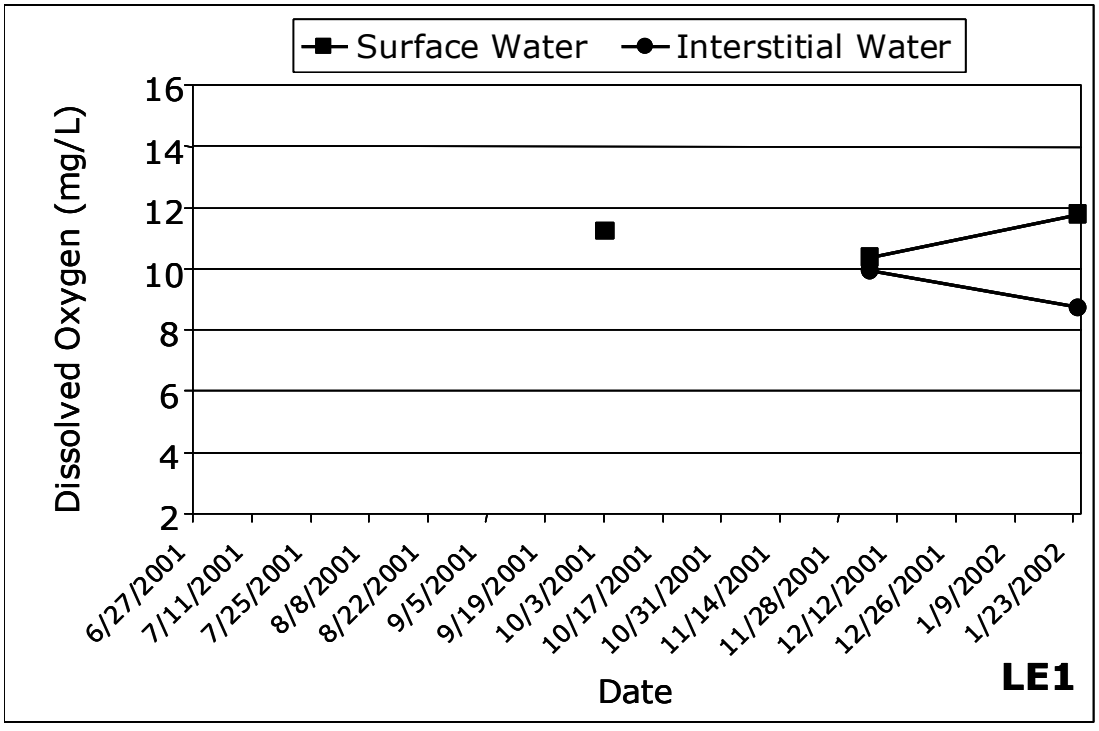

Surface Water
Interstitial Water
Dissolved Oxygen (mg/L)
16
14
12
10
8
6
4
2
6/27/2001
7/11/2001
7/25/2001
8/8/2001
8/22/2001
9/5/2001
9/19/2001
10/3/2001
10/17/2001
10/31/2001
11/14/2001
11/28/2001
12/12/2001
12/26/2001
1/9/2002
1/23/2002
Date
LE1

Table 12. Differences in dissolved oxygen (DO) concentrations between surface water and substrate interstitial water at sites in Big Spring Creek and Letort Spring Run.

Site	Date	Difference (mg/L)	Site Mean Difference
BS0	11/1/2001	0.63	0.32
	12/5/2001	0.13 *	
	1/24/2002	0.21 *	
BS1	11/1/2001	1.28	0.72
	12/5/2001	0.67	
	1/24/2002	0.20	
BS2	6/28/2001	5.20	4.18
	11/1/2001	4.55	
	12/5/2001	3.21	
	1/24/2002	3.74	
BS3	6/28/2001	4.10	4.38
	11/1/2001	3.40	
	12/5/2001	4.86	
	1/24/2002	5.14	
LE0	11/1/2001	1.41	1.90
	12/5/2001	0.82	
	1/24/2002	3.48	
LE1	12/5/2001	0.45	1.77
	1/24/2002	3.08	

* Interstitial water DO was greater than surface water DO, possibly due to groundwater upwelling, as observed on 10/30/2001 near the interstital water sampling probe.

Biochemical oxygen demand

BOD_5 results for 6 June 2001 were as follows: BS0 = 4.79 mg/L, BS1= 3.79 mg/L, BS2= 15.9 mg/L, BS3= 20.4 mg/L. The reason for this increase in BOD with downstream sites is unknown. Increased BOD does not necessarily indicate impairment of a stream; it is important only when it results in septicity, depressed DO, turbidity, or other negative consequences (McKee and Wolf 1963). As a point of reference, data collected by the Pennsylvania Department of Environmental Protection (DEP) indicate that BOD of 0.6 mg/L is often sufficient to cause impairment of macroinvertebrates in limestone streams (M. Embeck, DEP, personal communication). Biotic responses generally are detectable

when BOD reaches 2 mg/L and are almost always observed at 4 mg/L (S. Means, DEP, personal communication). Wohnsiedler (1969) considered 2 mg/L BOD_5 to represent a polluted area in Spring Creek, another Pennsylvania limestone stream (Wohnsiedler 1969). Surface water DO measurements taken in the early morning on the same day that the BOD_5 samples were collected exhibit a decrease at downstream sites BS2 and BS3 relative to the upstream sites in Big Spring Creek, suggesting that the elevated BOD_5 at downstream sites might have been depleting surface water DO in June 2001. The greater surface water DO concentration at BS3 relative to BS2 can be attributed to oxygenation of the water at the mill dam just upstream of BS3.

The increase in BOD_5 with distance from the hatchery discharge and the lesser BOD value for BS1 relative to BS0 suggest that the hatchery discharge was not responsible for the increased BOD_5 levels, or at least that there were other modifying factors. In fact, the lesser BOD_5 observed at BS1 relative to BS0 might indicate that the spring source provided a relatively larger contribution of biodegradable organic material than did the hatchery effluent in June 2001. Alternatively, toxic material(s) in the effluent might have inhibited bacteria in samples from BS1, thus decreasing their capacity to consume oxygen in the BOD_5 test and producing an underestimate of biochemical oxygen demand. The finding of less diversity in the microbial community at BS1 relative to BS3 supports the latter hypothesis (see **Microbial biofilm on substrates, *Light Microscopy***); however, that finding was based on comparison of only two samples. A comparison of chemical oxygen demand (COD, not measured) with BOD_5 would further assist in determining whether toxic inhibition was a factor. Finally, the increase in water temperature with distance downstream might result in increasing production of autochthonous organic material at downstream sites, thus increasing BOD_5.

BOD_5 data reported by Embeck (2000) for a period of lower fish production at the Big Spring hatchery (April, 2000) also showed a pattern of slight increase in BOD_5 at downstream sites on Big Spring Creek, but the BOD_5 values and the magnitude of increase were much smaller than those reported here. BOD_5 values for June 2001 far exceed the values reported by Embeck (2000) for February 2000, when fish production was high. However, our measurements were taken during summer when stream productivity and BOD would be expected to be increased relative to the winter and spring conditions represented by Embeck's data.

Total dissolved gases

Data collected with the saturometer were as follows: temperature= 11.1 °C, total gas pressure (saturometer reading)= 110.4 mm Hg, barometric pressure (BP)= 745 mm Hg, ΔP= 80 mm Hg. DO measured with YSI Model 95 DO meter was 9.59 mg/L. Results of calculations with GASWORKS.BAS are reported in Table 13. The total dissolved gas saturation at BS0 was 114.8 percent, and nitrogen saturation was 121.9 percent. ΔP is the total amount of gas dissolved in water, or percent saturation of gases in water (Boyd *et al.* 1994). ΔP greater than 0 indicates supersaturation of gases in water (Hargreaves and Tucker 1999). Allowable ΔP values vary with fish species, fish size, exposure period, culture conditions, and relative partial pressures of the dissolved gases present (Boyd *et*

al. 1994). The criterion listed in the Gold Book (U.S. EPA 1986c) for protection of freshwater and marine aquatic life is total dissolved gas concentrations in water not to exceed 110 percent of the saturation value for gases at the existing atmospheric and hydrostatic pressures. A threshold level when significant mortality begins to occur in juvenile chinook salmon and steelhead trout was reported to be 111 percent total gas saturation (115 percent nitrogen saturation) (U.S. EPA 1986c). Previous studies indicate that mortality increases with increasing percent nitrogen, even when total dissolved gas pressure is unchanged (U.S. EPA 1986c). The total dissolved gas saturation and nitrogen saturation values at BS0 indicate a significant risk of mortality to fish that remain in the affected area. The ratio of oxygen %:nitrogen % at BS0 is low, suggesting a potentially greater risk to fish than total gas pressure alone would indicate (Boyd *et al.* 1994). Clinical signs of gas bubble trauma include disequilibrium; gas bubbles in eyes, skin, or fins; exopthalmia ("pop-eye") or hemorrhaging around the eyes; and stress-induced secondary bacterial or fungal infections (Hargreaves and Tucker 1999). We are not aware of any reports of these clinical signs occurring in fish in Big Spring Creek. However, these signs might not be apparent, and gas bubbles may appear and disappear quickly (Hargreaves and Tucker 1999). Some fish can detect gas supersaturation conditions and avoid them when it is possible to move away or to swim to greater depth where hydrostatic pressure reduces the effects (Boyd *et al.* 1994). Some species of trout can detect and avoid supersaturated water, and others might not (U.S. EPA 1986c). Small fish in shallow water are particularly vulnerable to gas bubble trauma, which can result in coagulation of yolk in yolk-sac fry and serious or fatal injury due to blocked blood circulation in fry or young fish (Hargreaves and Tucker 1999). According to the Gold Book (U.S. EPA 1986c), "Juvenile salmonids subjected to sublethal periods of exposure to supersaturation can recover when returned to normally saturated water, but adults do not recover and generally die from direct and indirect effects" and "Research collected by Bouck *et al.* (1975) showed that gas supersaturated water at and above 115 percent total gas saturation is acutely lethal to most species of salmonids..." On the basis of this one measurement, it seems that dissolved gases will limit the ability of trout to spawn, or even to survive, very near to the Big Spring. Black and Macri (1997) previously reported elevated CO_2 concentrations near the Big Spring and to the end of the ditch; this suggests that elevated dissolved gas concentrations may be a continuing problem and might persist to the downstream end of the ditch.

Table 13. Results of saturometer calculations from data collected at the spring source (site BS0), Big Spring Creek, 27 February 2002.

Results from GASWORKS4.BAS	
Barometric Pressure (mm Hg) for #1 =	745
Water Temperature (°C) for #1 =	11.1
Salinity (ppt) for #1 =	0
Saturometer Reading (mm Hg) for #1 =	110.4
Oxygen Concentration (mg/L) for #1 =	9.59
Saturometer Calculations Results:	
Bunsen Solubility Coefficient =	0.0372
Water Vapor Pressure =	9.90 mm Hg
Total Dissolved Gas Pressure =	855.4 mm Hg
Oxygen Pressure =	137.0 mm Hg
Nitrogen Pressure =	699.8 mm Hg
Nitrogen + Argon Pressure =	708.5 mm Hg
Total Hyperbolic Pressure =	110 mm Hg
Oxygen Hyperbolic Pressure =	-17.0 mm Hg
N_2 + Argon Hyperbolic Pressure =	127.4 mm Hg
Total Percent Saturation =	114.8%
Oxygen Percent Saturation =	89.0%
Nitrogen Percent Saturation =	121.9%
Nitrogen Concentration =	21.2 mg/L
Ratio of Oxygen % vs Nitrogen % =	0.730

Other water quality parameters

Water pH fell within the optimum range for brook trout (6.5 – 8.0) at all sites and sampling times, with the exception of two measurements taken at BS3, and all pH measurements fell within the tolerance range for brook trout (4.0 – 9.5) (Raleigh 1982). Alkalinity measured in June and October was, as expected, moderate to high; high alkalinity usually promotes brook trout productivity (Raleigh 1982).

In June 2001, nitrite concentration in surface water was less at BS1 than at BS0 and increased with distance downstream from BS0 (Table 5). The trend in nitrite concentrations in interstitial water followed the trend in surface water concentrations. In October 2001, nitrite concentrations were less than the MDL of 0.013 mg NO_2-N/L at BS0, BS1, LE0, and LE1, and nitrite concentration increased with increasing distance downstream in Big Spring Creek (Table 8). Taken together with the ammonia measurements from the same days, these findings indicate that the hatchery discharge was not a significant source of nitrite to Big Spring Creek. Additional nitrite inputs to the creek may occur downstream of BS1, or ammonia from the hatchery discharge might be converted to nitrite at downstream sites. Although salmonids are among the fish species most sensitive to nitrite toxicity (Lewis and Morris 1986), few studies have assessed the chronic toxicity of nitrite to salmonids. Westin (1974) recommended 0.12 ppm NO_2

(0.037 ppm NO_2-N) as a maximum allowable level of nitrite in freshwater and suggested that 0.012 ppm NO_2 might be a safer choice. Russo *et al*. (1974) investigated the acute toxicity of nitrite to rainbow trout of different sizes and reported a 96-hr LC50 of 0.19 mg NO_2-N/L for the most sensitive group (average weight 11.9 g) (Russo *et al.* 1974). The minimum tested concentration that produced no mortalities, in rainbow trout of average weight 235 g, after exposure periods of 96 hr and 24 hr was 0.06 mg NO_2-N/L; lesser concentrations were not tested (Russo *et al.* 1974). In another study, nitrite exposure produced a 96-hr LC50 of 0.11 mg NO_2-N/L for the most sensitive size class of rainbow trout (average 15.3 g) that were tested (Russo *et al.* 1981). In a 1986 review of the toxicity of nitrite to fish (Lewis and Morris 1986), the authors concluded that that there was no evidence at that time that a nitrite concentration ≤ 10% of the 96-hr LC50 would cause harm to freshwater fishes. Ten percent of the least 96-hr LC50, reported by Russo et al., is 0.011 mg NO_2-N/L. This value is less than the MDL for our analytical method for nitrite, so all samples with detectable concentrations of nitrite exceeded it, including the sample from BS0 on June 28. Although this means that it is possible that the trout in Big Spring Creek are suffering slight, chronic effects due to nitrite exposure, there is some evidence to suggest that salmonids exposed to nitrite can develop resistance to it over time (Lewis and Morris 1986). This is a consideration for any future fish stocking efforts that might make use of non-resistant fish. It seems unlikely that nitrite toxicity is significantly impairing the trout fishery at Big Spring Creek. However, although observed concentrations are small, the toxicity of nitrite is exacerbated by low DO concentrations, since nitrite-induced methemoglobinemia reduces the capacity of the blood to transport oxygen (Lewis and Morris 1986). The limited information available indicates that salmonid species differ little in their susceptibility to nitrite toxicity (Lewis and Morris 1986).

Microbial biofilm on substrates

Heterotrophic plate counts

Mean heterotrophic plate counts were consistently lower at the BS1 site than at all other sites after both incubation periods (Table 14, Figure 6). However, differences in plate counts among BS0, BS1, and BS2 are so slight that they should be considered insignificant. Counts from the BS3 site were greater than counts from BS0, BS1, and BS2. Overall, no consistent differences among colony types or diversity were observed among sites. All plates contained many yellow or orange, spreading colonies that resembled representatives of the genera *Flavobacterium* and *Cytophaga*. These are common aquatic bacteria that use a variety of carbon compounds for energy. The only notable difference was the presence, on set of plates from sites BS1 and BS2, of a bright green, spreading colony type. Cells from this colony type had identical microscopic morphology to cells from the orange or yellow, spreading colonies (very small, Gram-negative rods less than 1 micron in width and diameter).

Although heterotrophic plate counts were consistently lower at the BS1 site than at all other sites, these differences were not statistically significant due to small sample sizes. According to M. A. Bruns (PSU, personal communication), regardless of statistical

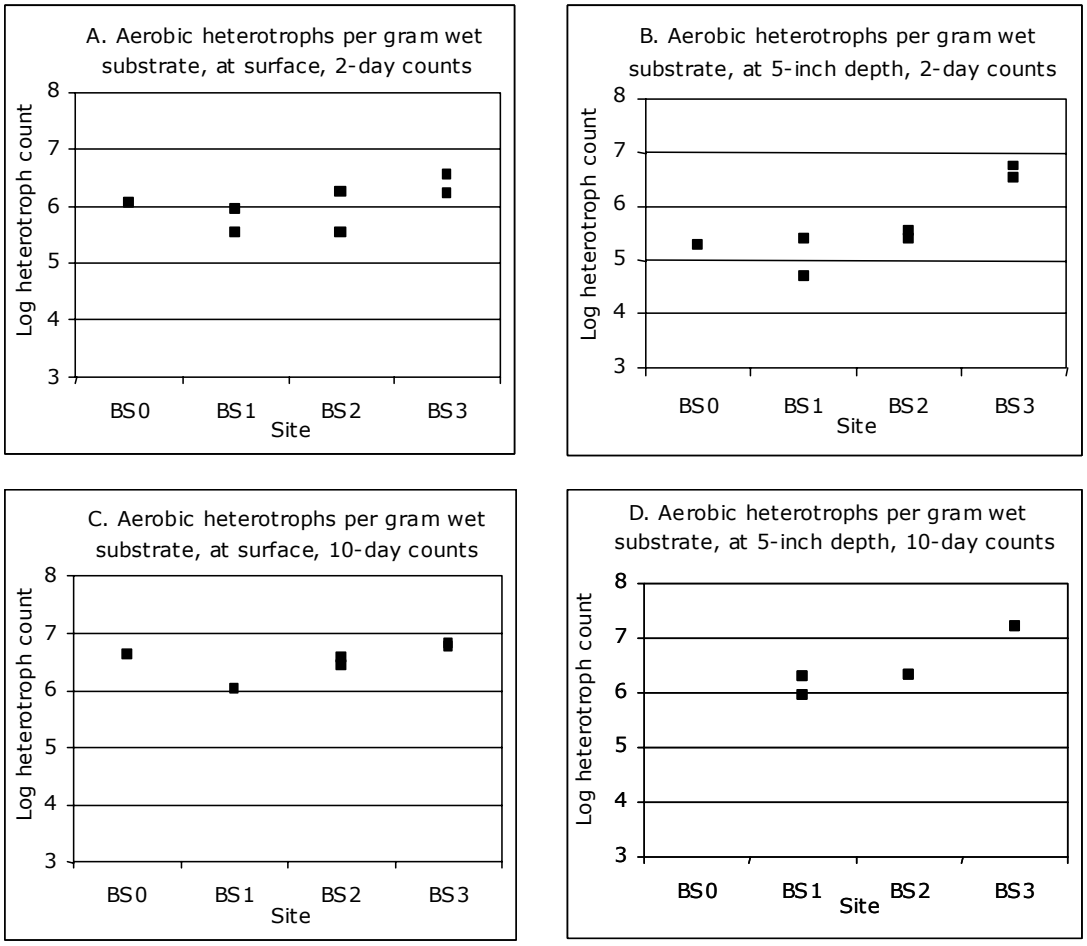
significance, the differences were not biologically meaningful, and all counts observed can be considered typical for aquatic systems. The significance of the bright green, spreading colony type in counts from substrate samples at BS1 and BS2 is not known. Polluted water generally results in increased HPC because it provides nutrients necessary for bacteria to thrive. The pattern of HPC among Big Spring Creek sites seems to be similar to patterns of phosphorus concentrations and percent organics in substrates, i.e., the numbers of heterotrophic organisms seem to vary with available nutrients.

Table 14. Mean aerobic heterotroph counts per gram wet sediment.

Sample	2-day counts	10-day counts
BS0 at surface	1.1×10^6 (n=1)	4.3×10^6 (n=1)
BS1 at surface	6.1×10^5 (n=2)	1.1×10^6 (n=2)
BS2 at surface	1.1×10^6 (n=2)	3.2×10^6 (n=2)
BS3 at surface	2.6×10^6 (n=2)	6.1×10^6 (n=2)
BS0 at 5 inches	1.9×10^5 (n=1)	ND (spreader)
BS1 at 5 inches	1.5×10^5 (n=2)	1.4×10^6 (n=2)
BS2 at 5 inches	2.9×10^5 (n=2)	2.2×10^6 (n=1)
BS3 at 5 inches	4.5×10^6 (n=2)	1.7×10^7 (n=2)

ND= non-detect due to early rapid growth of a few spreading colonies, which can affect outgrowth of other colonies and cause 10-day counts to be lowered artifactually.

Figure 6. R2A heterotrophic plate counts for substrate samples collected from Big Spring Creek on 28 June 2001.



Light microscopy

Some fresh substrate material was examined briefly under a light microscope. Due to time limitations, only three samples were examined— one from BS1 at the surface, one from BS1 at 5-inch depth, and one from BS3 at the surface. No unusual microbes were observed, nor did there appear to be any unusual overgrowth of biofilm on the samples. The prominent presence of diatoms, which are usually indicative of relatively unpolluted water, was noted in surface substrate samples from BS1. However, the diatoms in the sample were dead. Many live diatoms were present in surface samples from BS3, and the microbial community appeared to be more diverse as evidenced by the presence of spirochaetes, protozoans, and filamentous green algae. Little microbial life was observed in the BS1 sample collected at 5-inch depth, but this was expected based on observations from healthy stream systems elsewhere (R. Unz, PSU, personal communication). Since the sample from BS1 was collected earlier in the day than that from BS3, it is not clear

whether any significance can be attached to the live/dead state of the diatoms. It is possible that the longer storage time for the sample killed the diatoms in the BS1 sample.

Scanning electron microscopy

No evidence of biofilm overgrowth was observed in any samples. As with the light microscopic examination, the prominent presence of diatoms was noted, but the density of diatoms did not appear to be so great as to be a likely cause of substrate cementation.

Accumulation and consolidation of fine material in substrates

Others have observed that the substrate in Big Spring Creek is embedded (Embeck 2000; R. Schott, DEP, personal communication) or compacted (Black and Macri 1997). While this condition appears to have improved at the time when the current study commenced, it remains apparent that trout would experience difficulty in displacing these sediments to construct redds. There are many potential causes for the observed condition (variously termed 'embeddedness,' 'compacted,' 'colmation,' or 'cementation') of the substrate in Big Spring Creek. According to Brunke and Gonser (1997), "Colmation...includes all processes leading to a reduction of pore volume, consolidation of the sediment matrix, and decreased permeability of the stream bed." The nature of material(s) responsible for the embedding phenomenon, and in particular whether it is inorganic or organic material, might suggest the source(s) of the material and might have implications for efforts to restore the fishery.

The observation of large numbers of isopods (*Asellus*) indicates organic pollution. An informal qualitative assessment reveals that isopods are present in particularly great density just downstream of the hatchery discharge near our site BS1. The dominant presence of pollution-tolerant invertebrates downstream of the hatchery discharge was noted previously (Black and Macri 1997). Effluents from concentrated aquaculture facilities might increase the organic load in an aquatic system directly by addition of organic solids and/or indirectly by addition of nutrients and organics that stimulate productivity within the system (U.S. EPA 2002). When organic loading exceeds the capacity of an aquatic system to flush or degrade the material, organics can accumulate in stream substrates, producing anoxic conditions, increased toxic ammonia concentrations, and a foul odor (Embeck 2000).

Excessive nutrient loads in aquatic systems may cause algal blooms or overgrowth of aquatic macrophytes. The resultant increased photosynthetic activity can cause large diel cycles in dissolved carbon dioxide concentration and pH, producing conditions conducive to biogenic calcium carbonate precipitation and substrate colmation for part of the day (Cicerone *et al.* 1999). Biogenic calcium carbonate deposition has been observed in another Pennsylvania limestone stream (Spring Creek, Centre County, PA) due to overgrowth of aquatic macrophytes and algae stimulated by municipal sewage effluent, and deposition (marl) appeared to be worst in spawning riffles (Wohnsiedler 1969). Decomposition of organic matter such as a fish carcass also can cause calcium carbonate concretions to form under anoxic conditions (Berner 1968). Non-biogenic calcium

carbonate precipitation may occur where springs emerge from the ground, thus producing marl cementation.

The hypothesis has been advanced that a biofilm may be involved in the cementation phenomenon at Big Spring Creek, in part based on an observation of extensive microbial growth on substrate particles collected from Big Spring Creek (M. Embeck, DEP, personal communication). Biofilms may concentrate phosphorus to form phosphatic cements in estuarine sediments (Braithwaite and Gribble 1998). Fish hatchery effluents often contain elevated concentrations of nutrients including phosphorus (U.S. EPA 2000b). Organic pollution may produce an overgrowth of *Sphaerotilis*, a common filamentous bacterium, which then may carpet the substrates of receiving water bodies at the point of wastewater discharges (R. Unz, PSU, personal communication). *Sphaerotilis* also sticks to the surfaces of invertebrates and increases adhesion of inorganic particles, thus reducing gill function (Waters 1995a). These examples of synergism between inorganic and organic pollution suggest that a number of different factors may be implicated in the cementation of substrates and in producing negative impacts on the benthic community and fish production at Big Spring Creek.

Texture of stream substrate

Two primary factors control survival-to-emergence for salmonids (Lotspeich and Everest 1981). First, velocity of water through substrate interstitial spaces in redds must be sufficient to deliver an adequate supply of oxygen to incubating embryos and alevins. Second, substrate interstitial spaces in redds must be large enough to allow alevins to emerge. Sediment texture influences pore size and permeability of spawning gravels, which in turn control the velocity of water movement through spawning gravels and the movement of alevins during emergence (Lotspeich and Everest 1981). The percentage of spawning gravels composed of fines less than or 0.85 mm or 1 mm in size is often considered in determining whether salmonids can successfully reproduce using a particular substrate (Waters 1995b). Fines <0.8 to 1 mm in size reduce the flow of oxygenated water into redds and are likely to cause significant mortality of salmonid embryos when they constitute 20% or more of the total mass of the substrate (Waters 1995b). Although percent fines is a useful predictor of salmonid reproductive success, pore size and permeability are determined largely by the size distribution of grains in a sample and are directly related to mean grain size (Lotspeich and Everest 1981). Geometric mean particle size (GMPS) describes the central tendency of sediment particle size, and the sorting coefficient describes the distribution of grain sizes (Lotspeich and Everest 1981). The fredle index integrates both a measure central tendency and a measure of distribution in a single index that is obtained by dividing the GMPS by the sorting coefficient. Pore size and relative permeability increase with increasing fredle index.

Figure 7 and Table 15 present data used to characterize the texture of substrates in redds distributed along Big Spring Creek. Redds demonstrated a general trend toward increasing percentage of fines <1 mm, < 0.85 mm, and < 0.063 mm; decreasing GMPS; and decreasing fredle index with increasing distance downstream from the spring source.

This trend might suggest that input of fines occurs to an increasing extent with distance downstream, possibly due to runoff or erosion, or that input occurs upstream and fines settle out downstream or are gradually flushed downstream over time. However, the low gradient of Big Spring Creek would not favor flushing of deposited fines. A criterion of 20% fines less than 0.8 mm has been accepted by many researchers as the criterion above which significant mortality of salmonid embryos may be expected, although some laboratory studies have demonstrated a significant reduction in survival-to-emergence for salmon embryos at 10% fines < 0.8 mm (Waters 1995b). Percent fines < 1 mm ($\bar{0}$ = 25, n= 20) collected from trout redds ranged from 9.3 to 46, and percent fines < 0.85 mm ($\bar{0}$ = 23) ranged from 7.8 to 45. Of twenty redds sampled for substrate texture characterization, twelve contained substrates with more than 20% fines < 1 mm (or < 0.85 mm), and two redds (# 4 and #12) contained more than 40% fines. All but two redds (both 10 yd downstream of the old mill dam structure at the end of the ditch) contained more than 10 percent fines < 1 mm or < 0.85 mm.

There is some evidence that accumulation of fines in spawning gravels will have a lesser impact on survival of eggs and fry belonging to smaller salmonids like the brook trout. In a study of the characteristics of brook trout redds, the particle size of substrate forming the egg pockets was smaller than that typically found in the egg pockets of larger salmonids (Snucins *et al.* 1992). Larger eggs (of larger fish species) may require more passage of water through redds, and larger alevins may require larger sediment pore sizes for emergence (Kondolf 2000). For example, steelhead trout demonstrate a greater tolerance of fine material in spawning gravels than do chinook salmon, which produce larger eggs and fry (Tappel and Bjornn 1983). Negative population level effects may be incurred for brook trout when the percentage of fines less than 0.063 mm in size exceeds 0.6 – 1.0% of total spawning substrate (Hakala 2000). The percentage of fines < 0.063 mm was not greater than 0.1 for any redd sampled at Big Spring Creek and was not greater than 0.2 for any potential spawning site.

Measures of central tendency such as GMPS and fredle index might be better predictors of trout embryo survival than percentages of fines in substrates (Waters 1995b). However, substrates with different size class distributions (Tappel and Bjornn 1983) and wide variation in fine sediment content (Lotspeich and Everest 1981) may have the same GMPS, so the value of this measurement for comparison among studies is limited (Tappel and Bjornn 1983). In substrates collected from redds, GMPS ($\bar{0}$ = 5.3, n= 20) ranged from 2.0 mm to 11 mm, and fredle index ($\bar{0}$ = 1.6, n= 19) ranged from 0.53 to 3.3. Steelhead and chinook salmon embryos reared in artificial gravel mixtures with GMPS greater than 10 mm had survival rates of approximately 90%, and survival declined rapidly with decreasing GMPS less than 10 mm (Tappel and Bjornn 1983). A spawning substrate with GMPS of approximately 11-12 mm would be expected to allow 50% survival of salmonid embryos, based on combined survivals of coho salmon, sockeye salmon, steelhead trout, and cutthroat trout (from Shirazi and Seim 1979) (Waters 1995b); most biologists probably would consider a redd with 50% or greater emergence to be productive (Kondolf 2000). With the exception of redd #19a, samples of substrate material from all redds had GMPS less than 10, indicating that survival-to-emergence would be poor. Witzell and Macrimmon (1989) reviewed mean substrate size criteria for

brown trout based on substrate samples collected at redds and reported criteria ranging from 6.9 to 85.7 mm (Table 3). These authors also suggested a mean substrate size criterion of 5.7 mm for brook trout based on data that they had collected. The majority of substrate samples collected from redds in Big Spring Creek did not meet this criterion. Criteria for ranges of GMPS values also are provided in Table 3; however, the relationship of the criteria for mean particle size listed in Table 3 to embryo survival or survival-to-emergence has not been established in those studies. Spawning gravels with fredle index of approximately 2 – 2.5 or 3.5 – 4 (estimated from chart) would allow 50% survival-to-emergence for steelhead trout and coho salmon, respectively (Lotspeich and Everest 1981). In a study of wild brown trout in Spring Creek, Centre County, PA (Beard 1990), embryo survival of 50% or greater was not precluded by substrate beginning with a fredle index of 4 or greater at the start of the incubation period and declining to approximately 3 near the end of the incubation period and a corresponding mean minimum intergravel DO generally at 5 ppm or greater. Survival generally was less for sites that did not maintain a mean minimum intergravel DO concentration greater than approximately 5 ppm. A minimum fredle index allowing high embryo survival was not identified because the relationship among fredle index, intergravel DO, and embryo survival was not consistent, so other factors appear to be important. However, many redds in Big Spring Creek contained substrates with fredle index less than 2, suggesting that 50% survival would not be achieved in those redds.

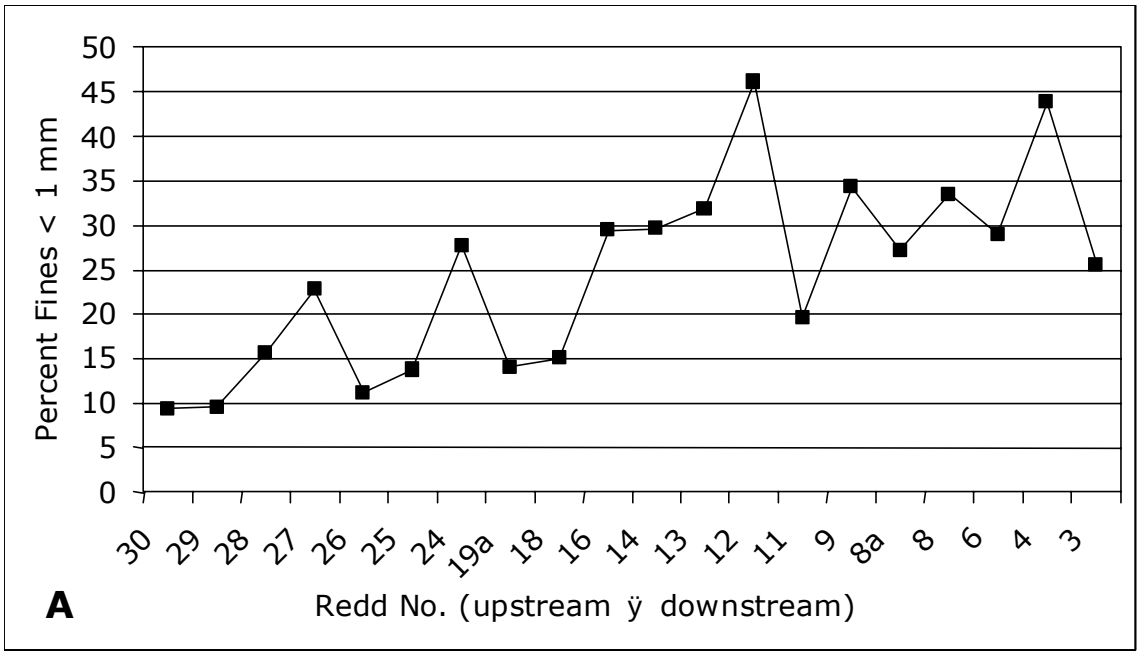
Figures 8 -10 and Table 15 present data used to characterize the texture of substrates in potential trout spawning areas near the interstitial water sampling probes, for comparison to substrate interstitial water quality. The sample for site BS1 was collected just downstream of Spring Road Bridge. For sites in Big Spring Creek, mean percent fines <1 mm ranged from 7.7 to 32, mean GMPS ranged from 3.3 mm to 17 mm, and mean fredle index ranged from 1.1 to 8.0. BS1 substrates had the greatest mean percent fines < 1 mm (< 0.85 mm and < 0.063 mm), the least mean GMPS, and the least mean fredle index for all sites, while BS0 substrates had the least mean percent fines < 1 mm (equal to LE1), the greatest mean GMPS, and the greatest mean fredle index for all sites. This pattern suggests that the hatchery is a significant source of fine material in the substrates at Big Spring Creek; however, fines originating from the spring source might settle out further downstream at BS1. LE1 substrates had the least mean fines < 1 mm (equal to BS0), < 0.85 mm, and < 0.063 mm. It was apparent that trout made heavy use of LE1 for spawning, based on our observations of dense, overlapping redds at that site. If trout use this same site annually, the females might have reduced the percentage of fines in the substrate with their repeated digging (Chapman 1988, Kondolf 2000). Upon visual inspection, substrates at LE0, upstream of the bridge at Bonnybrook Road, contained much more fine material, and far fewer redds were found at that location relative to LE1.

Despite the poor condition of potential spawning substrates at BS1 relative to other sites in Big Spring Creek, a substantial number of the observed redds (#26-32) were located near BS1. Substrates collected early in the spawning season from within redds near BS1 had lesser percentages of fines, greater fredle index, and greater GMPS than those collected from potential spawning locations at BS1. This difference may reflect the ability of female trout to improve substrate quality during preparation of redds. For redds

located between BS1 and BS2, however, substrate sampled from within redds was generally of lesser quality than that sampled from potential spawning sites near BS2 and more closely resembled the substrate of poorer quality collected at BS1. For redds between BS1 and BS2, redd cutting activity by females either did not substantially improve substrate quality, or the quality degraded more rapidly than it did at locations further upstream. The latter hypothesis is supported by the observed loss of redd structure over time, although it is not clear why improved substrate quality would be maintained to a greater extent at BS1 relative to locations further downstream.

Mean percent fines < 0.495 mm (< 0.50 mm) in substrates collected from potential spawning sites in Big Spring Creek were as follows: LE0 = 8.5, LE1 = 2.1, BS0 = 3.9, BS1 = 13, BS2 = 2.7, and BS3 (4.9). These results are different from results of previous assessments (Embeck 2000) of percent fines collected in sediment boxes at these locations, where mean percent fines < 0.5 mm (estimated from chart) were approximately 8 – 9 for Location 1 (≈ BS1) and Location 2, 12 – 13 for Location 3 (≈ BS2), and 5 for Letort 1 (≈ LE1). Embeck (2000) observed for locations in Big Spring Creek and Letort Spring Run a strong negative correlation between percent fines < 0.5 mm collected in sediment boxes and mean dissolved oxygen in interstitial water collected from adjacent sampling wells. In the current study, there were no significant correlations between interstitial DO and fredle index, GMPS, or percent fines < 1 mm, < 0.85 mm, or < 0.50 mm.

Figure 7. Percent fines less than 1 mm in size (Figure 7.A), geometric mean particle size (mm) (Figure 7.B), and fredle index (Figure 7.B) for substrate samples collected from trout redds in Big Spring Creek on 6 November 2001.



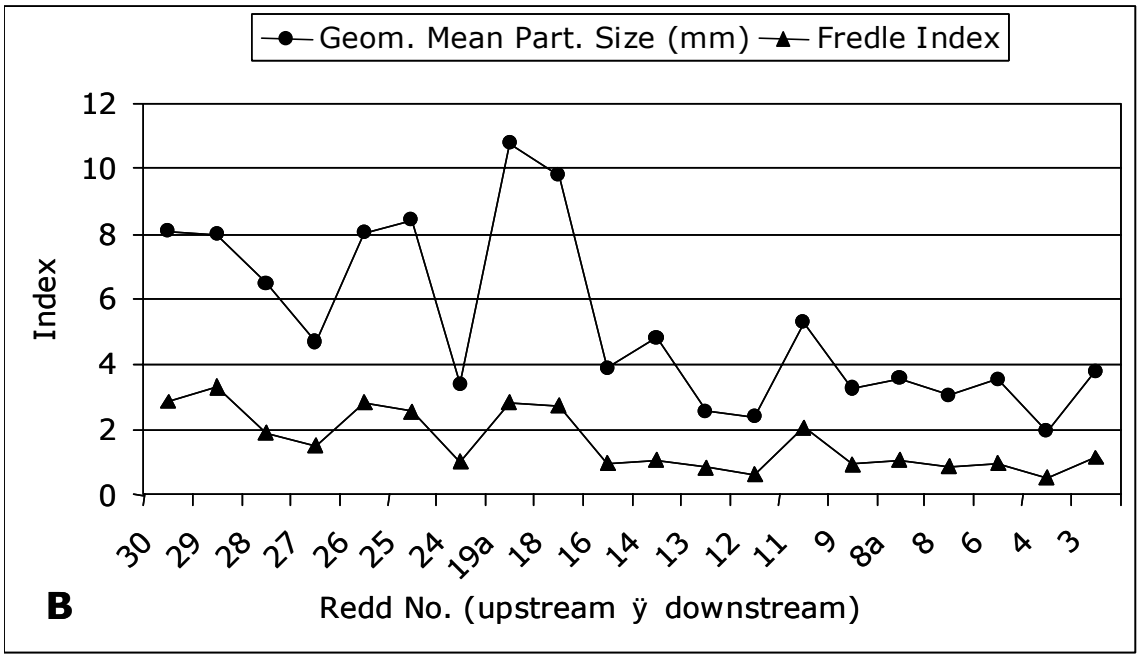


Table 15. Percent composition of fines less than 0.85 mm in size in stream substrate samples collected from trout redds in Big Spring Creek on 6 November 2001 (left) and from potential spawning sites in Big Spring Creek and Letort Spring Run on 10 December 2001 (right).

Redd no.	% Fines <0.85 mm
30	7.8
29	8.2
28	13
27	20
26	10
25	13
24	26
19a	13
18	14
16	28
14	28
13	28
12	45
11	18
9	30
8a	26
8	31
6	27
4	42
3	24

downstream

Site	% Fines <0.85 mm	Site Mean
LE0	10	
LE0	21	15
LE0	15	
LE1	3.0	
LE1	10	5.4
LE1	3.0	
BS0	9.2	
BS0	1.2	7.3
BS0	11	
BS1	26	
BS1	12	28
BS1	46	
BS2	10	
BS2	5.3	6.1
BS2	2.6	
BS3	12	
BS3	13	12
BS3	12	

Figure 8. Percent composition of fines less than 1 mm in size in stream substrate material (n= 3) collected from potential trout spawning sites in Big Spring Creek and Letort Spring Run on 10 December 2001.

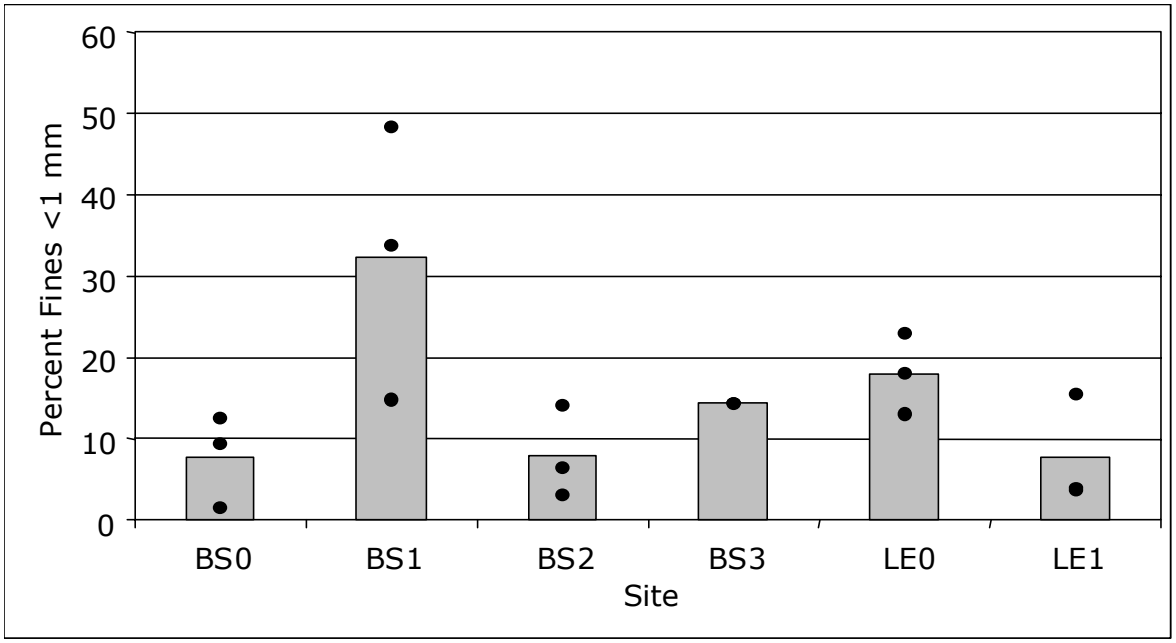


Figure 9. Geometric mean particle size (mm) of stream substrate material (n= 3) collected from potential trout spawning sites in Big Spring Creek and Letort Spring Run on 10 December 2001.

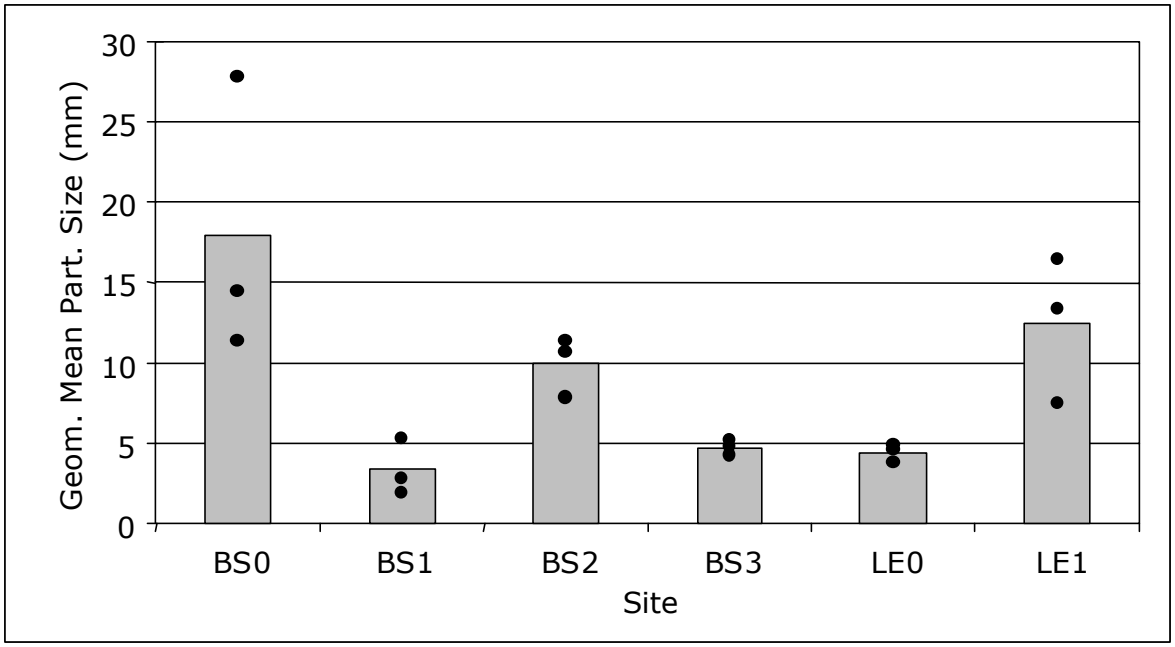
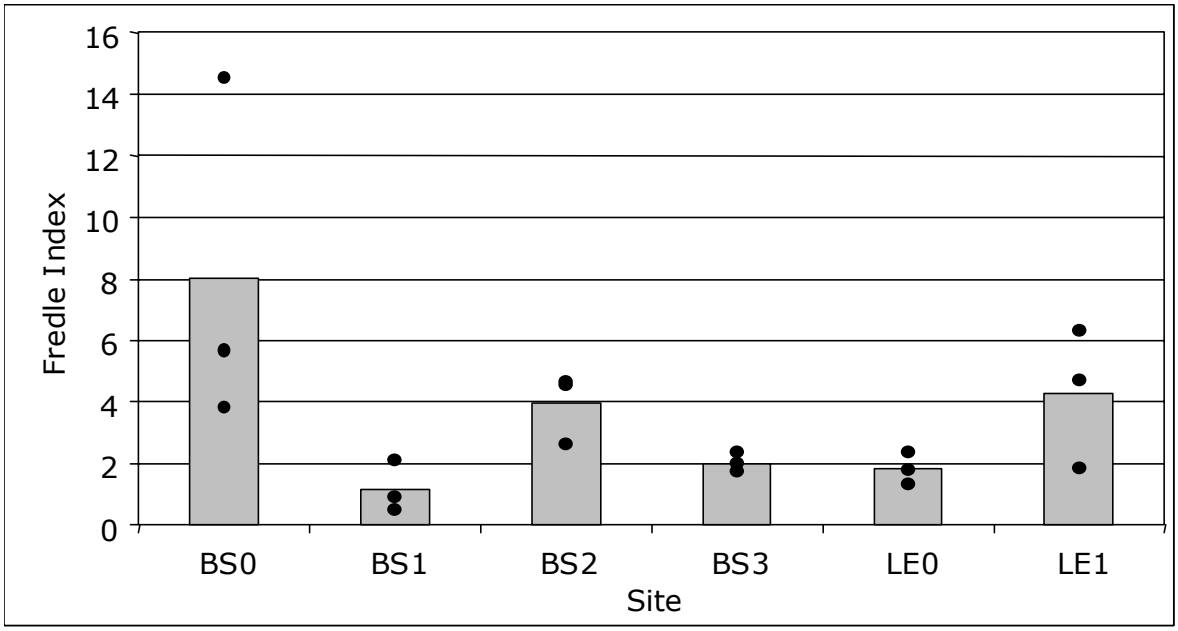


Figure 10. Fredle index for stream substrate material (n= 3) collected from potential trout spawning sites in Big Spring Creek and Letort Spring Run on 10 December 2001.



Calcite deposition in substrate

Because scanning electron microscopy (SEM) usage charges are expensive, initial examinations for calcite deposition were limited to substrate surface samples from BS1 and BS3. The BS1 samples were collected just downstream of the remains of the old mill dam structure at the lower end of "the ditch." These samples represent the worst-case for substrate consolidation based on previous reports, and this site receives the most immediate exposure to fish hatchery discharge. Substrate consolidation has not been observed at BS3. BS0 samples were not chosen as the reference material because calcium carbonate precipitation may take place at spring sources due to changes in pressure as the spring emerges from the ground. However, there currently is no reason to believe that this is happening at the Big Spring, and no substrate consolidation was observed there.

No evidence of secondary formation of calcite due to biogenic or physical processes was observed in any samples that were examined. Dropwise addition of 0.1 N hydrochloric acid onto substrate fines (<1 mm) failed cause evolution of carbon dioxide other than that which was obviously due to reaction with limestone chips originating from gravel used in the parking areas along Big Spring Creek.

Mineral precipitation modeling

Results of elemental analyses for surface water samples (Table 16) were used as input for the PHREEQC program (See page 11). Additional water quality data used as input were taken from Table 8.

Table 16. Concentrations of elements or chemical species measured in surface water samples from Big Spring Creek and Letort Spring Run, 3 October 2001.

	Concentration (mg/L) per Site			
Element	BS0	BS1	LE0	LE1
Al	0.08	0.09	0.24	0.07
B	<0.02	<0.02	<0.02	<0.02
Ba	0.04	0.04	0.05	0.05
Ca	48	49	64	62
Co	0.02	0.03	0.05	0.06
Cr	<0.02	<0.02	0.02	0.02
Fe	0.03	0.02	0.02	0.03
K	1.79	1.74	1.85	1.89
Mg	8.6	9.0	15.1	14.1
Mn	<0.02	<0.02	<0.02	<0.02
Mo	0.03	0.03	0.04	0.04
Na	4.4	4.9	3.77	3.86
Ni	<0.02	<0.02	<0.02	<0.02
Si	3.61	3.84	4.1	4.1
Sr	0.17	0.20	0.35	0.3 5
Ti	<0.02	<0.02	<0.02	0.02
V	0.02	<0.02	0.03	0.03
Zn	0.03	0.02	0.02	0.03
F	0.09	0.10	0.18	0.17
Cl	12.2	12.5	11.0	10.8
NO_2	<0.005	0.03	<0.005	<0.005
NO_3	19.5	20.0	27.3	26.5
PO_4	<0.02	<0.02	<0.02	<0.02
SO_4	12.0	12.0	19.3	18.6

In the PHREEQC calculation results, if the saturation index for a chemical phase is greater than 1, it is likely to precipitate out of solution. All sites examined (BS0, BS1, LE0, LE1) were comparable with regard to species that were likely to precipitate and the saturation indices for those species. Species likely to precipitate were oxy-hydroxides of

aluminum and iron, including gibbsite [$Al(OH)_3$], geothite [FeOOH], and iron (III) hydroxide [$Fe(OH)_3$]. Alunite [$KAl_3(SO_4)_2(OH)_6$] was likely to precipitate only near the spring sources at BS0 and LE0. LE1 was the only site that gave any indication that calcite [$CaCO_3$], aragonite [$CaCO_3$], or dolomite [$CaMg(CO_3)_2$] might precipitate. It is possible that some important ions were missing from the calculations, but the errors were within acceptable ranges for the model. These results indicate that calcite precipitation was not likely to occur under the conditions that existed at the time of sampling at any site other than LE1, where we observed no evidence of substrate consolidation and substantial evidence of trout spawning.

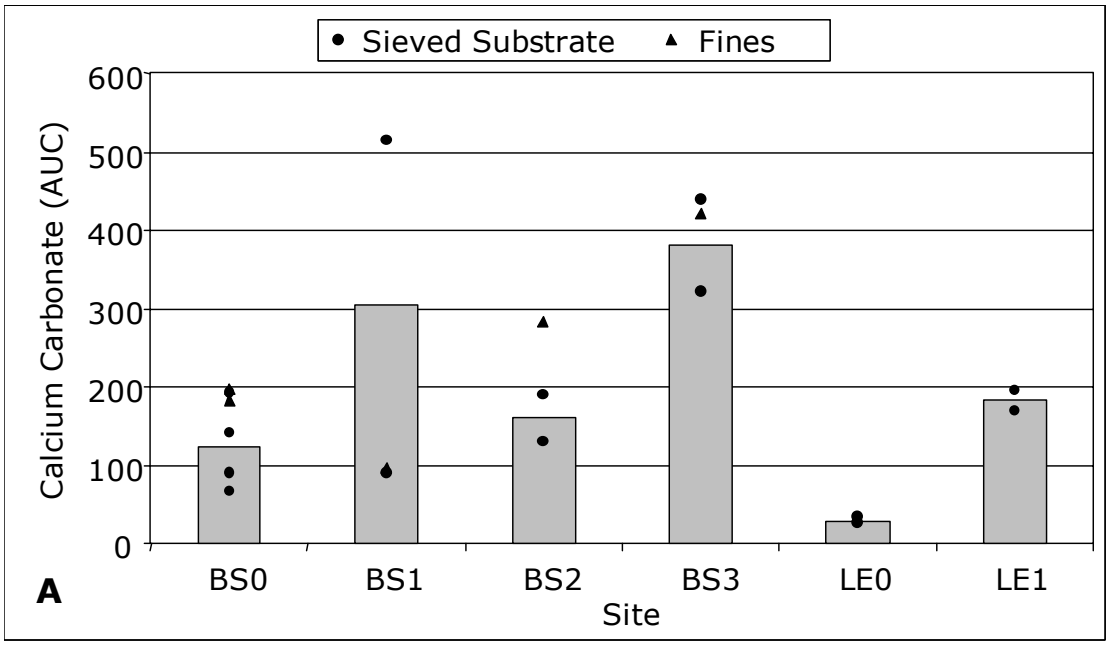
Crystal structure of minerals in fines

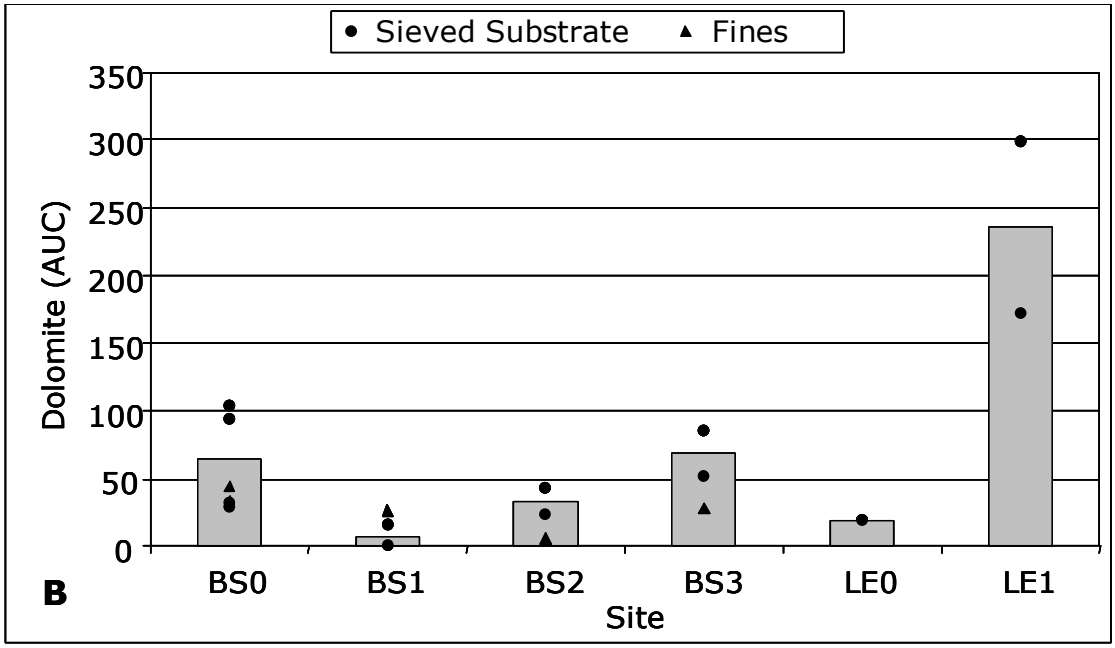
For all samples of substrate fines and clarifier sludge, the major mineral constituents were quartz, calcium carbonate, and dolomite (Figure 11). For each sample, the area under the curve (AUC) was calculated for the most intense peak on the x-ray diffraction spectrum for each major phase (quartz, calcium carbonate, dolomite). All data were first fitted with a Box Car curve algorithm to remove background. The K alpha 2 peaks were left intact, and a Pearson 7 algorithm was used to calculate the area under the peaks at approximately 26.6, 29.4, and 30.9 deg 2-theta, respectively. The AUC measurements were tabulated and then sorted from lowest to highest peak area for each phase. AUC values indicate the relative amount of the analyte that produced the peak. Precise quantitation is possible only with the use of standards, which substantially increase the cost for analyses. The goal in conducting these analyses was to attempt to identify significant differences in mineral profiles among samples rather than to quantify amounts precisely, so standards were not used.

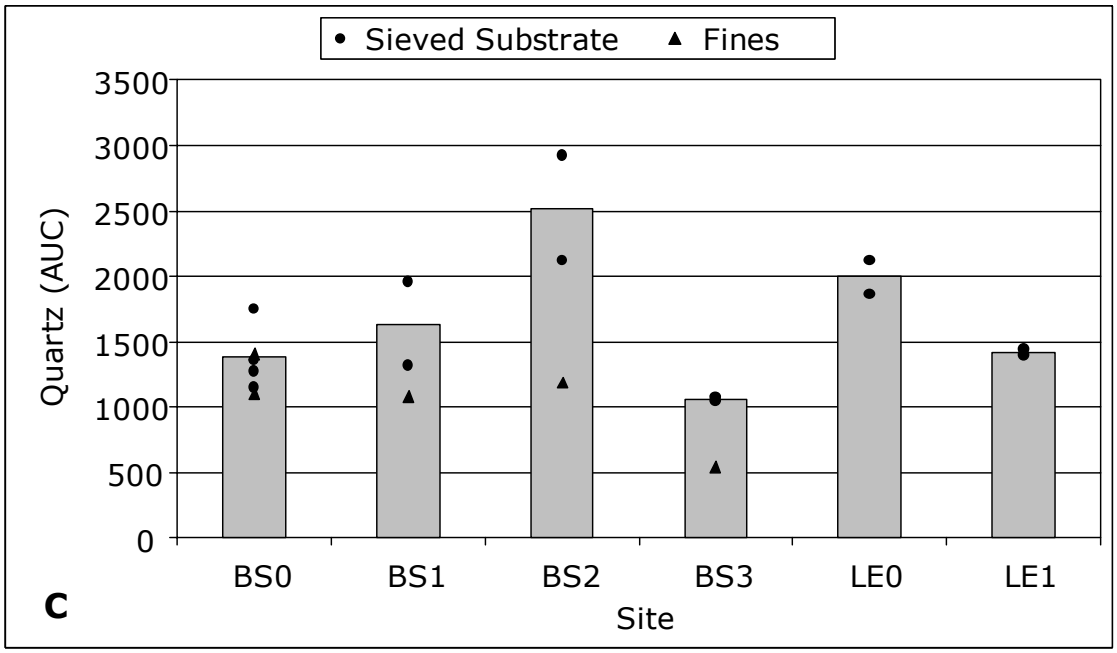
Calcium carbonate, dolomite and quartz are normal constituents of limestone stream substrates. Given that calcium carbonate is present in greater proportion in clarifier sludge than in stream substrates, it is possible that fines from the hatchery effluent are responsible for elevated calcium carbonate content at BS1 relative to BS0 and BS2. The reason for elevated calcium carbonate content of substrates at BS3 is not known, but this finding suggests that factors other than hatchery effluent may be responsible, at least in part, for the elevated calcium carbonate at BS1 as well. The pH of water is directly related to its acid content (typically carbonic acid). The solubility of calcium carbonate is directly affected by the pH of the water. If the pH of the water rises, calcium carbonate is forced out of solution and deposited in solid form. Gases in general, and specifically carbon dioxide, are less soluble in water at high temperatures. Therefore, as the temperature rises, the dissolved carbon dioxide decreases. This increases the pH of the water, reducing the solubility of the calcium carbonate and causing the mineral to deposit. This explains why calcium carbonate precipitates in a hot water heater. Diversion of cold water from the Big Spring through the fish hatchery increased the water temperature by as much as 5 °F on warmer days (D. Truesdale and T. Farner, PFBC, personal communication), possibly promoting the precipitation of calcium carbonate from the water.

The amount of calcium carbonate in fines from LE1 was similar to that found at BS2, and trout have no difficulty cutting redds at LE1. This suggests that compacted fine material, rather than calcium carbonate deposition, is the more likely cause of substrate cementation at BS2.

Figure 11. Results of x-ray diffraction (XRD) analysis of fines from substrate samples collected from Big Spring Creek (6 November 2001) and Letort Spring Run (9 November 2001). Only major constituents are reported (Figure 11.A. Calcium carbonate, Figure 11.B. Dolomite, Figure 11.C Quartz). AUC= area under the curve (instrument peak on a spectrum for the analyte), which indicates relative amount of substance that generated the peak. Samples sizes were: BS0 (n= 6), BS1 (n= 3), BS2 (n= 3), BS3 (n= 3), LE0 (n= 2), LE1 (n= 2). Circles represent data points for fines <1 mm sieved from the substrate. Triangles represent data points for fines collected from substrate surface accumulations without sieving. Shaded bars represent site means for sieved fines. Substrate surface fines were not collected from Letort Spring Run because large accumulations were not evident. For comparison, AUC for samples of sludge from the hatchery waste clarifier were as follows: calcium carbonate (479.30), dolomite (0.00), and quartz (124.80).




Sieved Substrate
Fines
350
300
250
200
150
100
50
0
Dolomite (AUC)
BS0
BS1
BS2
BS3
LE0
LE1
Site
B

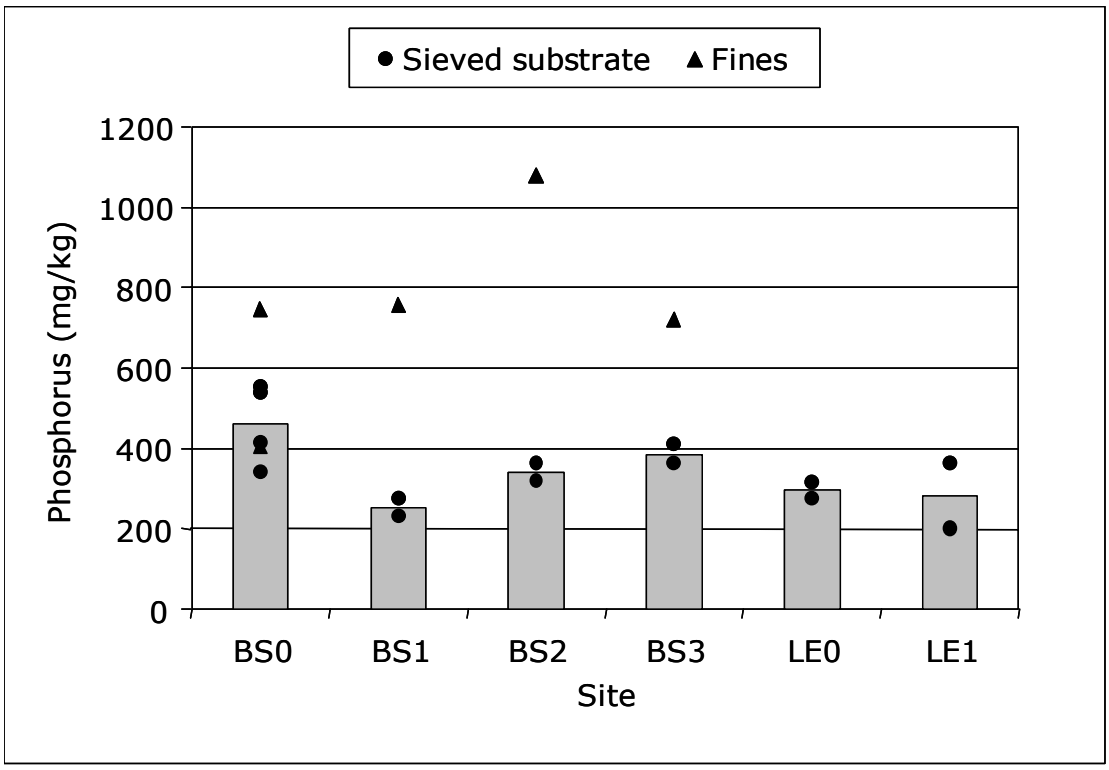

Sieved Substrate
Fines
3500
3000
2500
2000
1500
1000
500
0
Quartz (AUC)
BS0
BS1
BS2
BS3
LE0
LE1
Site
C

Phosphorus content of fines

Results of phosphorus content in substrate fines < 1mm are presented in Figure 12. Fish hatchery effluents often contain elevated concentrations of nutrients, particularly phosphorus (U.S. EPA 2000b). Biofilms can produce extensive polysaccharide films that concentrate phosphorus, producing phosphatic cements in estuarine sediments (Braithwaite and Gribble 1998). There was no identifiable trend in the phosphorus content of fines sieved from the substrates that would indicate that phosphate cementation was occurring due concentration of soluble phosphorus from the hatchery effluent by a biofilm. Mean total phosphorus content appeared to be greatest in sieved fines collected from BS0, upstream of the hatchery outfall, and increased progressively from sites BS1 to BS3. If substrate cementation occurred due to biofilm concentration of soluble phosphorus, the greatest phosphorus concentrations would be expected in fines sieved from substrates at BS1, where substrate cementation was most pronounced. Phosphorus content of substrate fines collected downstream of the fish hatchery discharge at BS1 was similar to that of samples collected from Letort Spring Run, a stream not affected by substrate cementation. The observed trend might indicate that there are sources of phosphorus input downstream from the hatchery discharge.

At all sites on Big Spring Creek, fine material that had accumulated on the substrate surface in slower moving portions of the stream contained greater phosphorus concentrations than did fines that were sieved from the substrate in portions of the stream with greater water velocity. The sample of surface fines with the greatest phosphorus content was taken from BS2; however, this comparison is based on a single sample of surface fines at most sites. Sludge from the fish hatchery waste clarifier, which was considered to be representative of solids that might enter Big Spring Creek via hatchery effluent, contained a high concentration of phosphorus (25806 mg/kg). It is possible that phosphorus associated with fine material released in the hatchery effluent may settle out only at the downstream sites, thus explaining the trend in phosphorus content of fines in the stream substrate and of fines collected from the surface of the substrate. Alternatively, sources of phosphorus input might occur downstream of the hatchery discharge.

Figure 12. Phosphorus concentrations (mg/kg) in samples of substrate fines (<1 mm in size) collected from Big Spring Creek and Letort Spring Run on 6 November and 9 November 2001. Samples sizes were: BS0 (n= 6), BS1 (n= 3), BS2 (n= 3), BS3 (n= 3), LE0 (n= 2), LE1 (n= 2). Circles represent data points for fines <1 mm sieved from the substrate. Triangles represent data points for fines collected from substrate surface accumulations without sieving. Shaded bars represent site means for sieved fines. Substrate surface fines were not collected from Letort Spring Run because large accumulations were not evident. For comparison, phosphorus concentration in a sample of sludge from the hatchery wastewater clarifier was 25,805 mg/kg.



Organic and carbonate content of fines

Given that percent organics in hatchery clarifier sludge was much greater than percent organics in any fines collected from Big Spring Creek substrates, one would expect that fines from BS1 would contained a greater percentage of organics than those from the other sites in Big Spring Creek if organic fines arising from the hatchery were depositing in the substrate at BS1. Our practice of sieving fine material from the substrates would have biased our results toward high estimates of percent organic content in stream substrates, since larger substrate material tends to consist of inorganic rock and pebbles. The percentage of organics in fines collected from both Big Spring Creek and Letort Spring Run, with the exception of fines from BS0, is low in comparison with streams considered to have high levels of organic pollution in the sediments (K. Henry, Dow Chemical Co., personal communication), particularly since our methods provided over-estimates of total substrate organic content. Organic content in substrates at BS1 and BS2 is less than that in substrates from all other sites, including those on Letort Spring Run, where no substrate cementation was observed (Figure 13). Taken together, these data indicate that organic solids are not contributing directly to the fine sediment load in Big Spring Creek. The pattern among sites for percent carbonate in fines sieved from stream substrates (measured by TGA) mirrors the pattern among sites for calcium carbonate content measured by XRD, except that calcium carbonate content at LE1 was more similar to that at BS1 than it was to that at BS2 (Figure 14). In June 2001, alkalinity of substrate interstitial water collected at BS1 and BS2 (where substrate cementation was observed previously) was substantially greater than alkalinity of surface water at those sites, whereas alkalinity of surface water and substrate interstitial water was equivalent at BS0 and BS3 and similar to alkalinity of surface water at BS1 and BS2. The alkalinity of interstitial water at BS2 was particularly great at 507.1 mg $CaCO_3$/L. The significance of the co-occurrence of these high alkalinity values with embedded substrates is not known. The water samples collected for analysis of alkalinity were not filtered, so it is possible that fine particulate carbonate material might be responsible for the high alkalinity value.

Figure 13. Percent organics in samples of substrate fines (<1 mm in size) collected from Big Spring Creek and Letort Spring Run on 6 November and 9 November 2001. Samples sizes were: BS0 (n= 6), BS1 (n= 3), BS2 (n= 3), BS3 (n= 3), LE0 (n= 2), LE1 (n= 2). Circles represent data points for fines <1 mm sieved from the substrate. Triangles represent data points for fines collected from substrate surface accumulations without sieving. Shaded bars represent site means for sieved fines. Substrate surface fines were not collected from Letort Spring Run because large accumulations were not evident. For comparison, percent organics in a sample of sludge from the hatchery wastewater clarifier was 14.39%.

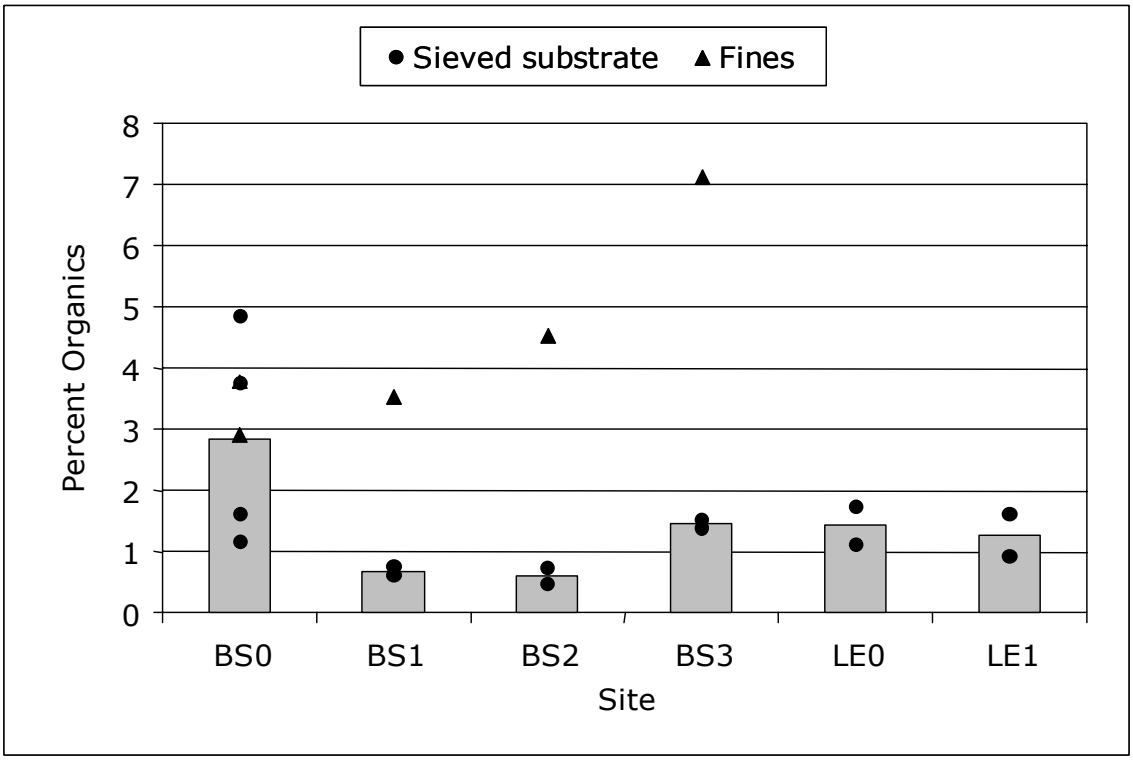
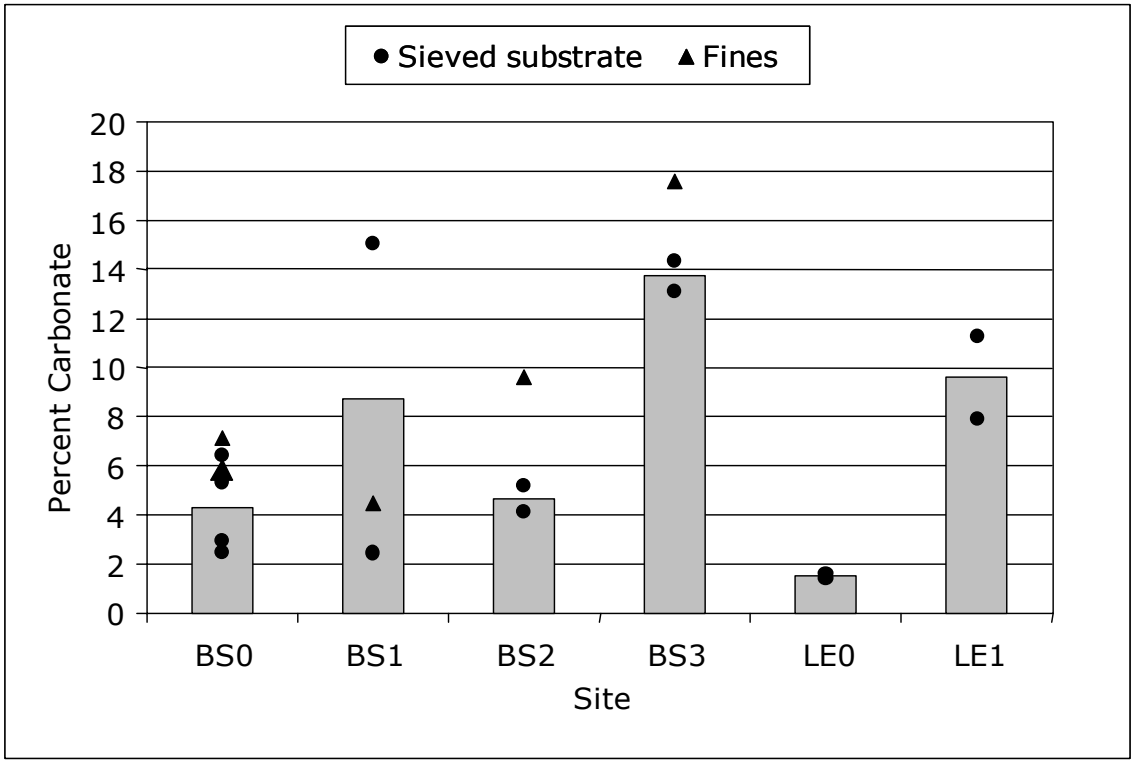


Figure 14. Percent carbonate in samples of substrate fines (<1 mm in size) collected from Big Spring Creek and Letort Spring Run on 6 November and 9 November 2001. Sample sizes were: BS0 (n= 6), BS1 (n= 3), BS2 (n= 3), BS3 (n= 3), LE0 (n= 2), LE1 (n= 2). Circles represent data points for fines <1 mm sieved from the substrate. Triangles represent data points for fines collected from substrate surface accumulations without sieving. Shaded bars represent site means for sieved fines. Substrate surface fines were not collected from Letort Spring Run because large accumulations were not evident. For comparison, percent carbonate in a sample of sludge from the hatchery wastewater clarifier was 21.44%.



CONCLUSIONS

In November, stream velocity and depth at all redds surveyed in Big Spring Creek (from the spring source downstream to the bridge at Nealy Road) met criteria for suitable spawning conditions for brown trout and brook trout. The stream reach from the stone bridge (approximately 1 km upstream of Newville on Spring Road) to Nealy Road had good depth and cover for trout, but very little gravel. By the end of February 2002, the typical winter die-back of the watercress beds resulted in wider channels with reduced stream flow, shallower depths, and reduced velocities at redd locations. Throughout the egg incubation period, ammonia concentrations in surface water and in interstitial water were sufficiently low for protection of early life-stages of salmonids in coldwater

systems. Water pH was within the optimum range for brook trout at all sites and sampling times, with the exception of two measurements taken at BS3, and all pH measurements fell within the tolerance range for brook trout. All surface water temperature measurements were within the tolerance range for brook trout, and most temperatures were within their optimal temperature range. Interstitial water temperatures were greater than the optimal temperature range for brook trout egg incubation prior to 24 January 2002 but were similar for Big Spring Creek and Letort Spring Run, a stream that supports a healthy reproducing trout population. It is possible that nitrite concentrations in Big Spring Creek are great enough to produce subtle non-lethal effects on trout. However, trout become acclimated to nitrite, and it is unlikely that any effects would negatively impact trout production.

Surface water DO was adequate for adult trout at all locations and sampling times. During the period when trout eggs and alevins were expected to be incubating in redds, interstitial DO concentrations at all sites other than BS3 exceeded the U.S. EPA coldwater 7-day mean criterion for protection of salmonid early life-stages. DO concentration in interstitial water at BS2 is a potential concern for several reasons. (1) Interstitial DO measured at BS2 on 30 October 2001 barely exceeded the criterion. (2) Our assessment was based on individual measurements rather than on 7-day means. (3) There is some evidence, based on data from this study and from a previous study by Black and Macri (1997), that DO concentrations near BS2 and BS3 decline at night, while the majority of our measurements were made during the day. If interstitial water levels also follow this diurnal pattern, it is possible that hypoxic conditions in the substrate are preventing trout from reproducing successfully at BS2. BOD_5 measured on 28 June 2001 was least at BS1, slightly greater at BS0, and increased markedly at BS2 and BS3. While increased BOD_5 appears to be associated with depletion of dissolved oxygen in the morning at BS2 and BS3, the source of oxygen-demanding substances is not clear, since BOD_5 was less at BS1 than at BS0. ORP measurements did not demonstrate a consistent trend that would identify a source of oxygen-demanding organic material, and the positive ORP values indicate predominantly aerobic conditions in both surface water and interstitial water at Big Spring Creek.

Based on a single measurement, total dissolved gas concentrations at BS0 are acutely lethal for most species of salmonids and would be expected to limit the ability of trout to reproduce in close proximity to the Big Spring. Additional measurements of total dissolved gases should be taken to determine whether gas supersaturation is a constant problem at the spring source and to document the distance that this condition persists downstream from the spring. Other measured water quality parameters such as temperature, pH, and alkalinity are unlikely to have a negative effect on trout production.

Counts of culturable heterotrophs from substrates in Big Spring Creek (heterotrophic plate counts) revealed no significant differences among sites that could be attributed to adverse effects of hatchery discharge. Examination of substrate material by light microscopy revealed no unusual microbes and no evidence of biofilm overgrowth. The microbial community appeared to be more diverse at BS3 than at BS1, but this assessment is based on comparison of only two samples, and the reason for this

difference is unknown. Examination of substrate material by scanning electron microscopy (SEM) yielded no evidence of biofilm overgrowth in any sample. The prominent presence of diatoms was noted with SEM, but the density of diatoms did not appear to be so great as to be a likely cause of substrate cementation.

It appears unlikely that phosphate cementation of the substrate was occurring due to concentration of dissolved phosphorus from the hatchery discharge into a biofilm because total P in fine material sieved from the substrate increased with distance from the hatchery discharge and was greater in fines collected at BS0 than in fines collected at BS1. It is possible that the hatchery discharge included high phosphorus fines that did not settle out until they reached BS2 or that there is another source of phosphorus upstream of BS2. Likewise, total phosphorus measurements in surface water suggest that the Big Spring may be a greater source of phosphorus than the fish hatchery discharge and that additional inputs of phosphorus may occur between BS1 and BS2. Substrate interstitial water contained much more total P than did surface water, and total P was elevated in interstitial water at BS1 and BS2 relative to BS3. This trend suggests that the hatchery discharge might be influencing phosphorus concentrations in interstitial water, but the Big Spring cannot be ruled out as the most important source or as a contributing source since total P was not measured in interstitial water at BS0. The trend in total P concentration in surface water on October 3 appears to mirror the trend in phosphorus concentration in fines collected from the surface of the stream substrate in November.

Results of a model for calcite precipitation and SEM examination of substrate fines for evidence of biogenic calcite formation do not support the hypothesis that calcite deposition is causing substrate consolidation. However, the extreme consolidation noted by others was not observed during the course of the current study. Based on results from X-ray diffraction analyses and thermal gravimetric analyses, fines sieved from the substrate at BS1 had elevated calcium carbonate content or carbonate content, respectively, relative to fines from BS0 and BS2. This condition might be due to deposition of solids from the hatchery, since clarifier solids contained a greater amount of calcium carbonate or carbonate than did stream substrates. The reason for elevated calcium carbonate or carbonate content of substrates at BS3 is not known, but this finding suggests that factors other than solids in hatchery effluent may be responsible, at least in part, for the elevated calcium carbonate at BS1 as well. The amount of calcium carbonate in fines from LE1 was similar to that found at BS2, and trout have no difficulty cutting redds at LE1. These data suggest that compacted fine material, rather than calcium carbonate deposition from solution, is the more likely cause of substrate cementation at BS2. Poor substrate texture, characterized by elevated percentages of fines, decreased geometric mean particle size (GMPS), and decreased fredle index, seems to be the most likely cause for substrate cementation at BS1 as well. Elevated carbonate or calcium carbonate was observed in fines sieved from the substrate at BS1, but there was no evidence of biogenic calcite deposition. Because hatchery clarifier sludge contains higher concentrations of carbonate or calcium carbonate, and because BS1 fines contain greater carbonate or calcium carbonate content than fines from either BS0 or BS2, it is likely that the source of the fines at BS1 is the hatchery discharge. However,

the even greater carbonate or calcium carbonate content of fines from BS3 indicates that there may be other causes for this phenomenon.

Big Spring Creek exhibits several features that increase the risk of sediment limiting salmonid reproductive success, including spring-fed hydrograph, sandstone or siltstone geology, low stream gradient, shallow and wide stream geometry, and lack of streamside forest and woody debris (Waters 1995b). It appears unlikely that discharge of organic solids from the hatchery is causing substrate cementation at BS1 or BS2. The degree to which the decomposition of organic matter in the substrates might contribute to depletion of DO in interstitial water is not known, but there does not appear to be a direct relationship between greater organic content in the substrate and decreased interstitial DO during the trout incubation period; site BS1 had the worst substrate texture but not the least interstitial DO concentration. Because calcium carbonate or carbonate content is elevated in fines sieved from stream substrates in locations of the stream previously identified as embedded or cemented, and percent organics is less at those locations relative to other locations at Big Spring Creek and Letort Spring Run, it appears that calcium carbonate is the more likely cause for cementation. However, the finding of similar or greater calcium carbonate or carbonate content at BS3 and LE1 relative to the BS1 or BS2 indicates that other factors may be responsible or might play an important role in causing this phenomenon. Because BOD_5 is excessively great in Big Spring Creek, it is possible that dissolved or suspended organic material is contributing to overgrowth of algae and aquatic macrophytes and thus indirectly contributing to deposition of fines in the stream. However, results of this study do not identify the source of the increased BOD. The data presented here suggest that inorganic sediment deposition is the most likely cause for decreased salmonid production in Big Spring Creek. Because the Big Spring Fish Culture Station was no longer in operation after 5 November 2001 and fish production had been cut back relative to previous years since July 2001 (J. Arway, PFBC, personal communication), we cannot know whether the presence of its effluent would have altered the results of this study.

ACKNOWLEDGEMENTS

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APPENDIX A

Comparison of total ammonia concentrations measured in Big Spring Creek in 2001 to U.S. EPA National Ambient Water Quality Criteria for total ammonia (U.S. EPA 1999).

Site - Sample	Date 2001	Temperature (°C)	pH	Total NH_3-N (mg N/L)	CMC[a]	CCC[b]
BS0 - surface	6/28	11.1	7.6	0.011	11.4	3.98
	10/3	11.0	7.0	< 0.006	24.1	5.91
	10/30	11.1	7.5	< 0.006	13.1	4.33
	12/5	11.0	7.0	< 0.006	24.5	5.95
BS0 - interstitial	10/30	13.5	7.8	< 0.006	7.5	3.03
	12/5	11.8	7.1	< 0.006	22.4	5.72
BS1 - surface	6/28	11.3	7.6	0.114	11.4	3.98
	10/3	12.1	7.3	0.093	17.1	5.01
	10/30	11.8	7.7	< 0.006	10.1	3.70
	12/5	11.2	7.0	< 0.006	23.3	5.82
BS1 - interstitial	6/28	12.3	7.6	0.053	11.4	3.98
	10/30	13.1	7.6	< 0.006	11.4	3.98
	12/5	13.5	7.2	< 0.006	20.6	5.50
BS2 - surface	6/28	12.0	7.7	0.026	9.6	3.58
	10/3	15.5	7.8	< 0.006	8.5	3.10
	10/30	11.6	8.0	< 0.006	5.6	2.43
	12/5	11.9	7.9	0.022	6.4	2.69
BS2 - interstitial	6/28	19.9	7.5	1.661	13.3	3.08
	10/30	14.9	7.9	< 0.006	6.8	2.73
	12/5	14.1	7.4	< 0.006	16.2	4.87
BS3 - surface	6/28	15.1	7.8	0.015	8.1	3.07
	10/3	17.1	8.4	< 0.006	2.8	1.17
	10/30	12.1	8.1	< 0.006	4.6	2.10
	12/5	11.7	7.6	0.032	11.4	3.98
BS3 - interstital	6/28	17.7	7.6	< 0.006	11.4	3.24
	10/30	13.9	7.8	< 0.006	8.1	3.18
	12/5	13.1	7.3	< 0.006	18.4	5.21
LE0 - surface	10/3	11.3	7.3	< 0.006	17.5	5.08
	10/30	11.9	7.6	< 0.006	11.4	3.98
	12/5	11.7	7.4	< 0.006	14.9	4.66
LE0 - interstitial	10/30	13.5	7.9	< 0.006	6.8	2.80
	12/5	12.4	7.4	< 0.006	15.3	4.73
LE1 - surface	10/3	13.1	7.7	< 0.006	9.3	3.50
	12/5	11.9	7.4	0.007	14.5	4.59
LE1 - interstitial	12/5	12.5	7.5	< 0.006	13.9	4.48

[a] pH-dependent Criterion Maximum Concentration (salmonids present)

[b] Temperature and pH-dependent Criterion Continuous Concentration (fish early life stages present)

Reference:

U.S. EPA. 1999. 1999 Update of ambient water quality criteria for ammonia. U.S. Environmmental Protection Agency, Office of Water. Washington D.C. EPA-822-R-99-014. 153 p.