PROJECT REPORT

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Title: EVALUATION OF NITRATE SOURCES IN SPRINGS OF THE SANTA FE RIVER BASIN USING NATURAL TRACERS: GEOCHEMICAL, SPECIFIC MICROBIOLOGICAL, AND MULTIPLE STABLE ISOTOPIC INDICATORS

Abstract:

Geochemical and microbiological methods were used to evaluate sources of nutrient enrichment, nutrient cycling, and groundwater residence times in two springs that discharge water from the karstic Upper Floridan aquifer to the Santa Fe River in northern Florida. Various chemical tracers (delta ^{15}N and ^{18}O isotopes of nitrate; dissolved gases, major ions; $^{3}H/^{3}He$, chlorofluorocarbons (CFCs), SF_6, selected pesticides and degradates; and selected organic wastewater compounds) were analyzed in water samples collected from Hornsby Spring (flow >2.8 m^3/s) and Poe Spring (flow 1.4-2.8 m^3/s), at three separate times during varied hydrologic conditions. Additionally, C and N stable isotopes were measured in algae, selected invertebrates, and fish from spring pools and runs. Nitrate-N concentrations were lower in water samples from Poe Spring (0.16-0.31 mg/L) than Hornsby Spring (0.45-0.52 mg/L). Denitrification likely has occurred in both spring systems, indicated by enriched delta $^{15}N\text{-}NO_3$ values (15.7-23.9‰), excess N_2 (1-2 mg/L), low dissolved oxygen (<0.5 mg/L), and elevated dissolved organic carbon (2.3-7.6 mg/L). $^{3}H/^{3}He$, CFCs, SF_6 concentrations were consistent with binary or exponential mixing models and indicated shorter groundwater residence times for Poe Spring (25 years) than Hornsby Spring (40 years). Fecal coliforms and human enterovirus sequences were detected in April 2004 samples compared to non-detects in previous samples (September and December 2003), although no corresponding differences were measured for chemical indicators. Delta ^{13}C and ^{15}N values for filamentous algae, invertebrates, and fish exhibited significant spatial and seasonal differences within and between spring systems. Algae delta ^{13}C and delta ^{15}N values were consistently depleted relative to water, ranging from -39 to -48‰, and 6 to 16‰, respectively. Invertebrates and fish were enriched in both ^{13}C and ^{15}N relative to algae, with the large variability in consumers expressed in ^{13}C from Hornsby Spring and in ^{15}N from Poe Spring. The insect repellent DEET was found in low micrograms per liter concentrations in both springs, but concentrations of 12 other organic compounds indicative of wastewater sources were very close to laboratory method reporting levels (<0.5 ug/L). Three pesticides (atrazine, metolachlor, and hexazinone) also were detected in Hornsby and Poe Springs at concentrations close to method reporting levels (<0.01 ug/L).

Geochemical Indicators:

Using chemical indicators to assess sources of nitrate contamination, and age of ground water discharging from Hornsby Spring and Poe Springs

Brian G. Katz

Springs provide not only sources of potable water, and sites of recreational, and cultural value, but they also afford a way to assess regional ground-water quality because spring waters integrate ground water vertically, spatially and temporally from large parts of an aquifer. In Florida, as in many parts of the world, agricultural activities and other land uses have impaired the quality of spring waters by contributing large quantities of nutrients to ground-water recharge (Dietrich and Hebert, 1997; Focazio et al., 1998; Burg and Heaton, 1998; Buzek et al., 1998; Böhlke, 2002). Nitrate-N concentrations in northern Florida spring waters have increased substantially from background concentrations in the UFA (≤0.1 mg/L; Katz, 1992) to more than 5 mg/L during the past 40-50 years (Hornsby and Ceryak, 1999; Katz et al., 1999). Large quantities of nitrate-N are being discharged from springs to surface waters (Pittman et al., 1997) and nuisance levels of algae and macrophytes occur in many spring-fed rivers (Jones et al., 1996; Hornsby and Mattson, 1998).

Protecting spring waters from further degradation requires information on contamination sources and transit times of ground water discharging from springs. Recent studies have used ground-water transit times derived from environmental tracers along with historical records of nitrogen loading to ground water, to better understand the timing of nitrate input to the subsurface and its movement through the ground-water system (Cook and Böhlke, 1999; Katz et al., 2001; Böhlke, 2002). Environmental tracer data have shown that transit times of ground water discharging from springs in karst systems can be highly variable (Dincer and Payne, 1971; Focazio et al., 1998; Plummer et al., 2001; Katz et al., 2001).

In response to human health and ecological concerns, the U.S. Geological Survey (USGS) in cooperation with the Alachua County Environmental Protection Department conducted a study of Hornsby Spring and Poe Springs to better understand sources of nitrate contamination, timescales of contamination of ground water, and sources of eutrophication in spring runs. This paper describes the use of a multi-tracer approach to better understand sources of nitrate contamination during varied flow conditions in two karstic springs. Ground-water dating techniques are applied by using chemical and isotopic tracers (CFCs, SF_6, ^3H and ^3He) along with lumped parameter flow models to assess timescales of nitrate contamination in ground water discharging from these large karstic springs. By evaluating different tracers simultaneously, important information can be gleaned about ground-water transit times and the age-frequency distribution for spring waters (Böhlke, 2002) that can be used for important resource-management decisions.

Description of Sampled Spring Systems

Water discharging from Hornsby and Poe springs originates from the upper Florida aquifer (UFA), which consists of sediments that range in age from the late Eocene to mid-Oligocene (Scott et al., 2002). Several climatic, geologic, and hydrologic factors control the amount and variability of ground water discharge from these springs. Dominant factors include the amount and frequency of rainfall and recharge, the porosity and permeability of the UFA, the size of the ground-water contributing area, and the hydraulic head distribution. Rainfall amounts vary considerably from year to year.

Porosity and permeability of the UFA vary greatly near both springs, because openings in the water-bearing limestone range from small solution openings along joints or bedding planes to large cavernous openings and networks of conduits developed in modern karst or paleokarst areas. Variability in aquifer permeability near spring conduit networks is reflected by a large range in transmissivity, from 2,800 to greater than 1.1×10^6 m^2/day (Bush and Johnston, 1988; Sepulveda, 2002).

Methods

Sample Collection and Analysis

Samples of water from the two springs were collected by lowering a positive displacement dual-piston pump head about 5-15 m into the spring vent. Water was pumped at approximately 0.06 L/s through 0.63-cm copper (refrigeration-grade) tubing. Specific conductance, pH, dissolved oxygen, and temperature were measured in situ by a multi-probe unit that was lowered directly into the spring vent. Field-sampling protocols, methods for chemical and isotopic analyses, are described in detail by Katz et al. (1999). Nitrogen and oxygen isotope ratios of nitrate are analyzed at the USGS Stable Isotope Laboratory in Reston, Virginia, using a new procedure that includes the bacterial conversion of nitrate to nitrous oxide and subsequent measurement on a continuous flow isotope ratio mass spectrometer (Revesz and Casciotti, 2003). Nitrogen isotope ratios are reported in parts per thousand (per mil) relative to N_2 in air (Mariotti, 1983) with a 2-sigma uncertainty of 0.5 per mil. Oxygen isotope ratios are reported in per mil relative to VSMOW reference water and normalized on a scale such that SLAP reference water is -55.5 per mil (Coplen, 1988, 1994). The 2-sigma uncertainty of oxygen isotope results of nitrates is 1.0 per mil.

Dating spring waters

Anthropogenic activities, such as atmospheric testing of thermonuclear devices and various industrial processes, have released ^3H, CFCs, and SF_6, into the atmosphere in low but measurable concentrations over the past several decades (fig. 1). Precipitation that incorporates ^3H, CFCs, and SF_6 from the atmosphere infiltrates into the ground and carries a particular chemical or isotopic signature related to atmospheric conditions at the time of recharge to the UFA. These dating methods presume that gas exchange between the unsaturated zone and air is fast, but that shallow ground water remains closed to gas (CFCs, SF_6, ^3He) exchange after recharge (Schlosser et al., 1989; Plummer and Busenberg, 1999; Busenberg and Plummer, 2000).

^3H and $^3H/^3He$ Age Dating

Information about ground-water transit times can be obtained by comparing measured ^3H concentrations in ground water with the long-term ^3H input function of rainfall measured at the International Atomic Energy Agency (IAEA) precipitation monitoring station in Ocala, Florida (Michel, 1989) (fig. 2). Atmospheric weapons testing beginning in the early 1950's increased ^3H concentrations in rainfall in this area to a maximum of several hundred TU during the mid-1960's, followed by a nearly logarithmic decrease in concentrations to the present. ^3H concentration is reported in tritium units (TU), with 1 TU equal to 1 ^3H atom in 10^{18} hydrogen atoms. Combined measurements of ^3H and $^3He_{trit}$ (radioactive daughter product of tritium decay) define a relatively stable tracer of the initial ^3H input to ground water, which can be used to calculate the $^3H/^3He_{trit}$ age from a single water sample (Schlosser et al., 1988, 1989; Solomon and Sudicky, 1991). $^3H/^3He_{trit}$ ages generally are not affected by contamination, sorption, and microbial degradation processes that can alter CFC or SF_6 concentrations (Plummer et al., 1998;

Busenberg and Plummer, 2000). The distribution of ^3H and $^3He_{trit}$ can, however, be affected by hydrodynamic dispersion and mixing of different age waters (Solomon and Sudicky, 1991, Reilly et al., 1994). Non-tritiogenic ^3He, which generally is negligible in a shallow aquifer with predominantly young water, is determined from analyses of ^4He and Ne in the spring water sample (Schlosser et al., 1988, 1989).

Water samples for the determination of $^3H/^3He_{trit}$, ^4He, and Ne were collected in pinch-off Cu tubes (10 mm diameter, 80 cm length, approximately 40 mL volume) while applying back pressure to the discharge from the sample tube to prevent formation of gas bubbles during sample collection. Samples were analyzed at the Noble Gas Laboratory of Lamont-Doherty Earth Observatory using quantitative gas extraction followed by mass spectrometric techniques (Schlosser et al., 1989; Ludin et al., 1998).

Chlorofluorocarbon and SF_6 Age Dating

The CFC and SF_6 dating techniques rely on the stability of these halogenated hydrocarbon and sulfur compounds (gases) in the hydrosphere, which has led to their effective use as tracers to age-date ground water recharged during the past 50 years (Plummer and Busenberg, 1999; Busenberg and Plummer, 2000). If the CFC and SF_6 concentrations in the aquifer have not been altered by biological or geochemical processes, and are of atmospheric source, apparent ages can be evaluated.

Apparent ages for CFCs and SF_6 are estimated based on the equilibrium partitioning between recharging ground water and the partial pressures of trichlorofluoromethane (CCl_3F, CFC-11), dichlorodifluoromethane (CCl_2F_2, CFC-12), trichlorotrifluoroethane ($C_2Cl_3F_3$, CFC-113), and SF_6 in the troposphere or soil atmosphere (fig. 2). Concentrations of CFCs and SF_6 in ground water are functions of the atmospheric partial pressures and the temperature at the base of the unsaturated zone during recharge. The recharge temperature and the quantity of dissolved excess air (Heaton and Vogel, 1981) are determined from gas-chromatography analyses of N_2 and Ar in the headspace of water samples collected in the field (Busenberg et al., 1993). An apparent age of the sampled water is determined from a comparison of the partial pressure of each CFC compound and SF_6 in the sample, calculated from their measured concentrations using solubility data for each compound, with the record of atmospheric partial pressures over North America at different times (fig. 1). Input functions for CFCs and SF_6 were obtained from their atmospheric input curves, and assuming a ratio of summer-to-winter infiltration coefficient of 1.0. Concentrations of the three CFC compounds and SF_6 ideally provide four independent ages, which can be used as a cross-check on the sampling and analytical methods, and used to evaluate mixing processes. Analytical procedures for CFCs and SF_6 are described by Busenberg and Plummer (1992) and Busenberg and Plummer (2000).

Estimating Age of Ground Water Discharging from Springs

The apparent age of ground water that discharges from springs is based on the piston-flow assumption. The piston flow model (PFM) assumes that after a tracer is isolated from the atmosphere (at the time of ground-water recharge), it becomes incorporated in a parcel of water that moves from the recharge area with the mean velocity of ground water. All flow lines are assumed to have similar velocities and hydrodynamic dispersion and molecular diffusion of the tracer are assumed to be negligible. For a PFM, the concentration of ^3H in a water parcel separated from the atmosphere since the time of recharge or entry into the ground-water system can be defined as (Zuber, 1986):

$$C(t_i) = C_{in}(t_i-\tau)\exp(-\lambda\tau) \qquad (1)$$

where $C(t_i)$ is the concentration at a given time, τ is the residence time of the water parcel in the aquifer, $C_{in}(t_i-\tau)$ is the input concentration at the time of recharge, and $\lambda = 0.05626$ (the decay constant of ^3H). For nonradioactive tracers in the PFM, such as CFCs and SF_6, $\lambda = 0$ and the concentration at any given time (t_i) is equal to the input concentration at time ($t_i-\tau$).

Results

Spring Water Chemistry

Water from both springs is a $Ca\text{-}HCO_3$ type with generally low concentrations of dissolved solids (320-460 mg/L) (table 1). Spring waters tended to be slightly oxic, with dissolved oxygen concentrations ranging from 0.31 to 1.4 mg/L for Hornsby Spring and from 0.27 to 0.50 mg/L for Poe Springs. Dissolved oxygen concentrations generally were lower in samples collected during this study compared to those in samples collected in 1998 (Katz et al., 1999). Water from Hornsby Spring had lower dissolved organic carbon concentrations (2.3-3.0 mg/L) than Poe Spring (2.6-7.6). Saturation indices (SI) with respect to calcite and dolomite typically were slightly less than zero for both spring waters, indicating that ground water is slightly undersaturated or near saturation with respect to these two minerals that compose the limestone rock matrix of the UFA.

Nitrate-N concentrations are elevated above background ground water concentrations in both springs. Nitrate concentrations in water from Hornsby Spring were consistent during the three sampling events and ranged from 0.45 to 0.52 mg/L; whereas nitrate concentrations in water from Poe Springs showed a larger fluctuation (0.16 to 0.31 mg/L) (table 1). Nitrate concentrations were lower in water samples collected from both springs during this study compared to samples collected in 1998. Nitrate concentrations in samples collected on August 21, 1998, were 0.80 and 0.82 mg/L for Hornsby Spring and Poe Springs, respectively.

Nitrogen and other stable isotopes

$\delta^{15}N$ values of nitrate in ranged from 16.8-18.3‰ for Hornsby Spring and from 15.7 to 23.9‰ for Poe Springs (table 2). These values are considerably higher than measured values for samples collected in 1998 for Hornsby (8.7‰) and Poe Springs (10.6‰). Some of this discrepancy could be related to the bacterial method for nitrate isotope measurements that may overestimate the nitrogen isotope ratio of atmospheric nitrate samples by as much as 1 to 2 per mil (Sigman and others, 2001) due to the enrichment in O-17 in atmospheric nitrate by mass-independent processes (Michalski and Thiemans, 2000). The higher values measured during this study likely are indicative of recharge of nitrogen from animal/human waste sources or possibly denitrification in the aquifer, which would result in enriched values of $\delta^{15}N$ (Hübner, 1986; Marrioti et al., 1988). Denitrification likely has occurred in both spring systems, indicated by the enriched delta $^{15}N\text{-}NO_3$ values (15.7-23.9‰), excess N_2 (1-2 mg/L), low dissolved oxygen (<0.5 mg/L), and elevated dissolved organic carbon (2.3-7.6 mg/L). In these karstic spring systems, it was assumed that denitrification would be negligible given that spring waters tend to be aerobic and contain very low dissolved organic carbon concentrations. However, it appears that denitrification is occurring or may have occurred sometime in the past.

Stable isotopes of oxygen ($\delta^{18}O$) and hydrogen (δ^2H) were used in this study to determine the origin of water and to characterize possible mixing of different sources of water in springs. Values of $\delta^{18}O$ and δ^2H are relatively constant for both springs during the three sampling periods, and are consistent with recharge from rainfall that has undergone little or no evaporation.

Apparent ages of spring waters

Apparent ages of spring waters are obtained by subtracting the piston-flow modeled recharge year (table 3) from the sample-collection year (2003 or 2004) based on the CFC and SF_6 data. For both springs, there was not much variation (considering uncertainty in methods) in apparent ages among the three sample times. However, concentrations of CFC-11, CFC-12, CFC-113, and SF_6 in spring-water samples generally did not yield concordant apparent ages, based on atmospheric equilibration data and the piston flow assumption. Water samples from Poe Springs have concentrations of CFC-11, CFC-12, CFC-113, and SF6 within the range expected for air-saturated waters that are less than 50 years old; but the apparent equilibration years are quite different for these compounds. For example, SF6 apparent ages for Poe Springs range from 10-14 years, but CFC apparent ages range from 27 years (CFC-12) to 48 years (CFC-11). Similar results were obtained for water samples from Hornsby Spring, although apparent ages are greater for Hornsby Spring than for Poe Springs. SF_6 apparent ages for Hornsby Spring range from 16-19 years, and CFC apparent ages range from 38-42 years (CFC-113) and from 35-44 years (CFC-11). Water samples from Hornsby Spring had CFC-12 concentrations that are higher than those possible for equilibrium with modern air and are termed “contaminated” (Table 3). Apparent ages estimated using CFC-11 concentrations generally are older than those obtained from CFC-12 and/or CFC-113 concentrations (Table 3). This may indicate that CFC-11 was preferentially degraded (Plummer and Busenberg 1999). However, if all three CFC compounds are presumed to be stable in the aquifer system, then the lack of concordance could result from complex mixtures of groundwater from shallow and deep flow paths that converge near the spring.

Younger SF_6 apparent ages relative to those estimated using CFC data may result from low levels of contamination (Busenberg and Plummer 2000; Zoellmann and others 2001). Two other springs in the Suwannee River basin, Troy Spring and Ichetucknee Blue Hole Spring, were contaminated with SF_6 from hydrologic studies in the early 1990s that introduced SF_6 as a tracer to measure ground-water velocities in nearby conduit systems.

However, when information for ^{3}Hand ^{3}He, CFCs, and SF_6 were compared with each other and with theoretical modeled curves for various flow scenarios, different ages or ground-water residence times were found for the two spring waters. Tracer concentration data were consistent with binary-mixing or exponential-mixing models and indicated shorter groundwater residence times for Poe Springs (25 years) than Hornsby Spring (40 years), as shown in figure 2. The older age for water from Hornsby Spring compared to Poe Spring is consistent with differences in the size of the ground-water contributing areas for the two springs and differences in spring-water chemistry. The estimated spring basin for Hornsby Spring is 360 km2 compared to that for Poe Spring (170 km2). The Hornsby Spring basin includes more of the Northern Highlands area and Cody Scarp, and subsequently areas where the Upper Floridan aquifer is confined by clays of the Hawthorn Group. This is reflected in differences in ground-water chemistry between Hornsby and Poe Springs. Higher median concentrations or magnesium, silica, sulfate, and dissolved solids all indicate that recharge to Hornsby Spring is moving through clay-rich material that comprises the Hawthorn Group sediments that are present in the Northern Highlands. Similar findings in the Lake City area by Lawrence and Upchurch (1976) also showed that elevated concentrations of magnesium, sulfate, and silica reflect the siliclastic nature of the Hawthorn Group sediments and higher degree of confinement of the Upper Floridan aquifer in the Northern Highlands.

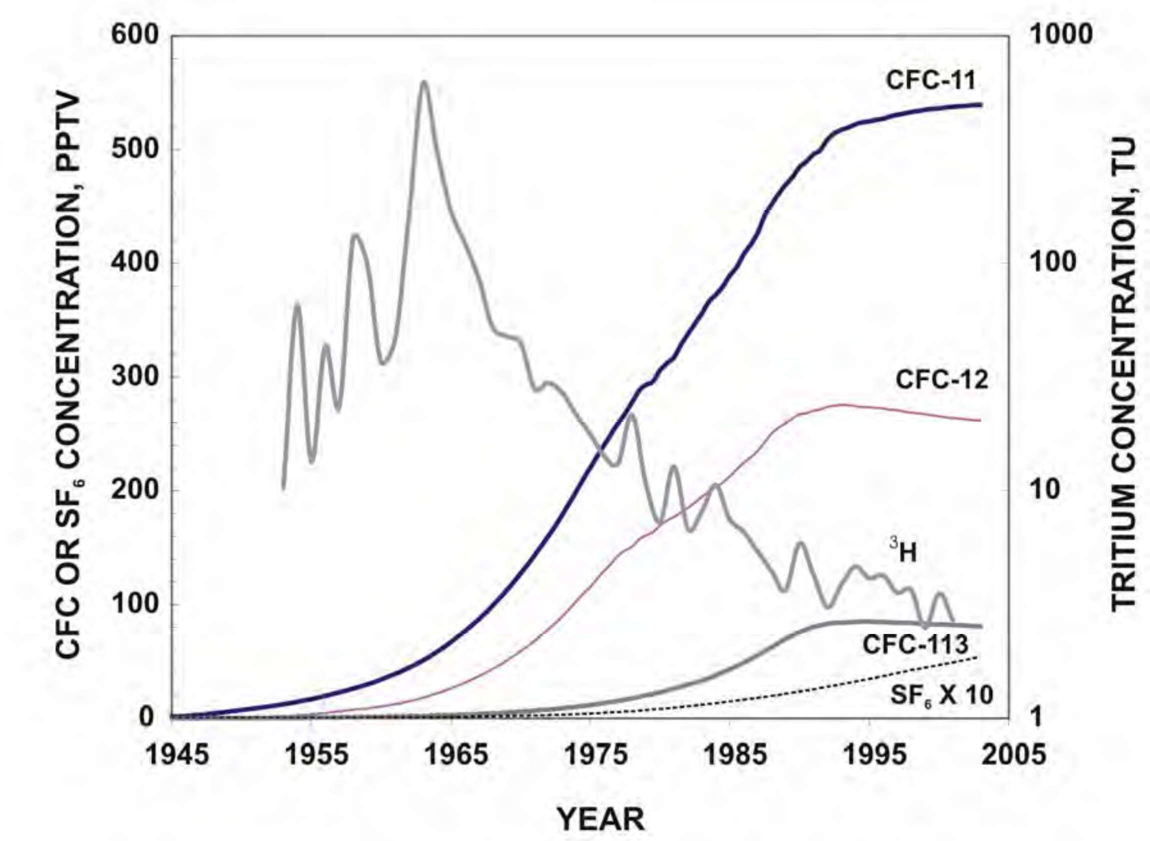

Figure 1. Input history of atmospheric concentrations of CFCs and SF_6 (Plummer and Busenberg, 1999: Busenberg and Plummer, 2000), and tritium (^3H) concentrations in rainfall collected at the IAEA station in Ocala, Florida.

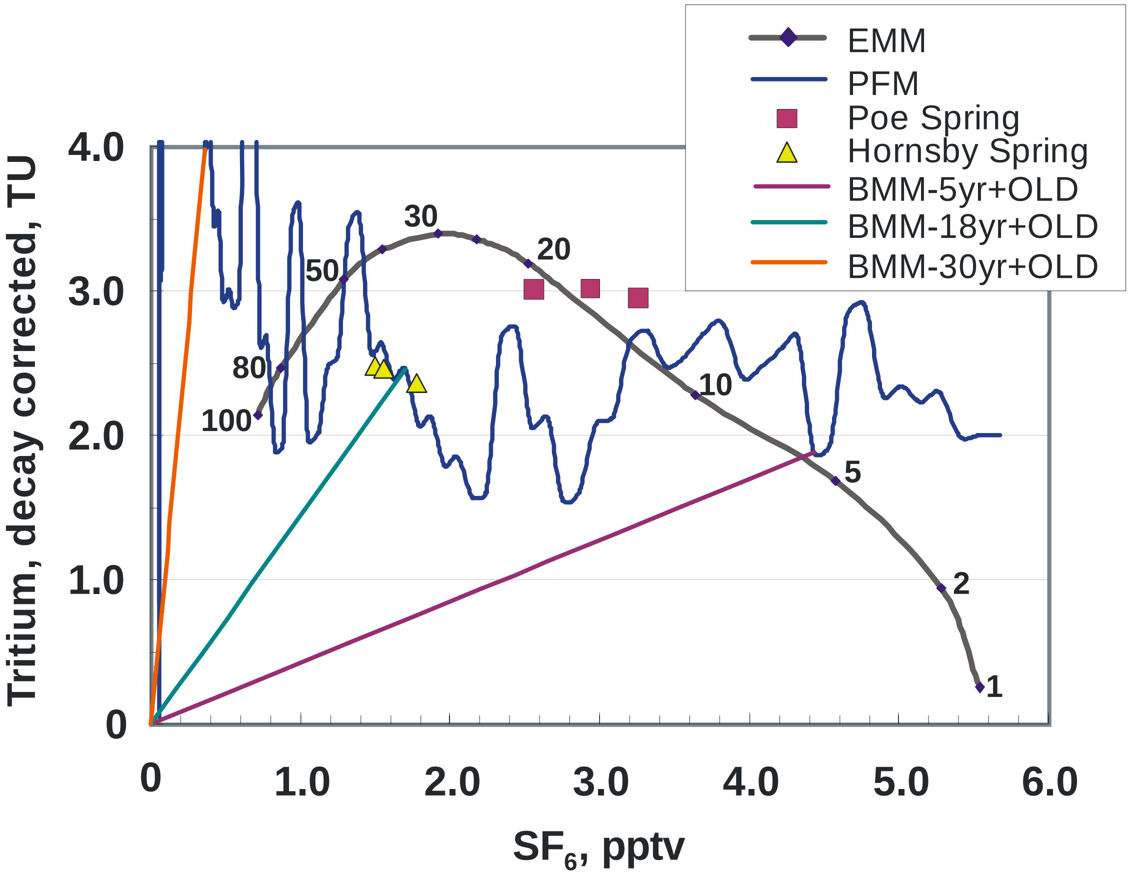

Figure 2. Comparison of SF6 and tritium concentrations in samples of spring water to theoretical output curves for different flow scenarios using lumped parameter models including exponential mixing (EMM), piston flow (PFM), and binary mixtures (BMM) of young water (5, 18, and 30 years) with old (tracer free) water (greater than 50 years old). Ages of water along EMM curve are shown.

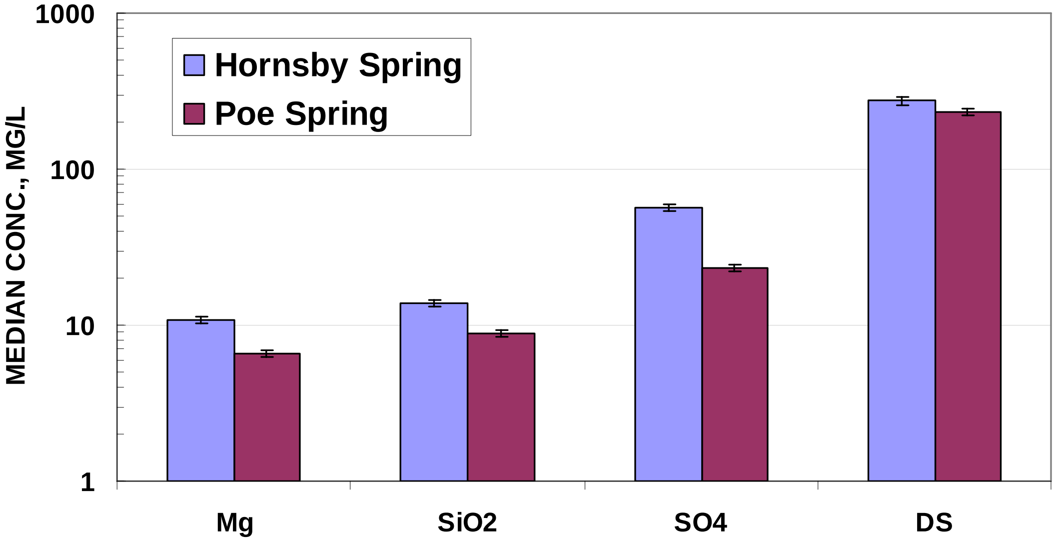

Figure 3. Differences in median concentrations of magnesium (Mg), silica (SiO_2), sulfate (SO_4), and dissolved solids (DS) between Hornsby and Poe Springs.

Contaminants of emerging concern--organic wastewater compounds and pesticides

Analyses were made for 67 organic wastewater compounds and over 70 pesticide compounds to determine if human wastewater and or agricultural activities were possible sources contributing contaminants to Hornsby and Poe Springs. The only pesticide compounds that were detected in these samples were atrazine, metolachlor, and hexazinone (table 4). All three pesticide compounds were detected at very low concentrations (generally less than the method detection limit of 0.013 micrograms per liter). Thirteen organic wastewater compounds were detected at very low concentrations in Hornsby and Poe Springs (table 4), the concentrations of most of these compounds were estimated values below the method reporting level but were quantifiable. DEET was the only compound detected above the method reporting level at concentrations of 0.6 to 2.5 micrograms per liter in Hornsby Spring and 0.2 to 7.5 micrograms per liter in water from Poe Spring. DEET has been found in other spring waters from northern and central Florida (Katz and others, 2005; Phelps, 2004).

Table 1. Physical properties and concentrations of major dissolved chemical species, and spring discharge for Hornsby Spring and Poe Springs.
[Concentrations are in milligrams per liter unless otherwise noted; DO, dissolved oxygen; SC, specific conductance; Q, discharge]

	T, ^{o}C	pH	SC, uS/cm	DO	Q, ft^3/s	Ca	Mg	Na	K	NH_4-N	Cl	SO_4	HCO_3	F	NO_3-N	PO_4-P	SiO_2	DOC	NH_4-N+or g-N	DS
Hornsby Spring																				
09/25/03	22.4	6.83	453	1.35	102	71	11.3	8.58	1.16	<0.04	13	58	190	0.3	0.45	0.08	13.3	3.0	0.12	271
12/17/03	22.4	7.47	402	0.67	61	78	10.9	8.93	1.15	<0.04	12	49	196	0.2	0.52	0.08	13.8	2.3	0.11	274
04/15/04	22.4	6.90	460	0.31	41	72	10.2	8.51	1.06	<0.04	13	56	195	0.2	0.47	0.08	13.9	2.6	<0.10	273
Poe Springs																				
09/24/03	22.4	6.50	317	0.27	82	64	6.54	7.31	0.94	<0.04	14	23	208	0.2	0.31	0.10	8.75	2.6	0.13	229
12/18/03	22.3	7.60	402	0.50	55	70	6.46	8.19	0.87	<0.04	13	21	213	<0.2	0.30	0.09	8.88	4.6	0.20	234
04/21/03	22.4	7.08	419	0.46	42	72	6.95	8.72	0.93	<0.04	14	24	211	0.2	0.16	0.10	9.78	7.6	<0.10	242

Table 2. Concentrations of stable isotopes in water from Hornsby Spring and Poe Springs.
[Concentrations are in per mil; see text for information on reference samples]

	δ^{13}C	δ^{2}H	δ^{18}O	δ^{15}N-NO3	δ^{18}O-NO3
Hornsby Spring					
09/25/03	-11.0	-14.4	-2.9	17.6	13.2
12/17/03	-11.2	-13.6	-2.9	18.3	14.7
04/15/04	-11.1	-14.7	-2.9	16.8	14.2
Poe Spring					
09/24/03	-11.6	-14.6	-3.0	15.7	13.5
12/18/03	-12.3	-11.0	-2.9	23.9	13.3
04/21/03	-11.9	-14.4	-2.9	21.5	13.8

Table 3. Concentrations of CFC-11, CFC-12, CFC-113, and SF6 in solution; calculated mixing ratio, and piston-flow model ages for sampled spring waters. [Amp. denotes ampule number; pg/kg, picograms per kilogram; Contam., CFC concentrations in water samples are higher than values in equilibrium with 2001 atmospheric concentrations; NP, age calculation not possible due to likely contamination; yrs, years before sample date; Modern, age is close to zero.]

Spring Name	Sampling Date	Recharge Temp, ^{o}C	Recharge Elev., m	Calculated Atmospheric mixing ratio in pptv			Concentration in solution, pg/kg			Excess Air mL	SF6 conc. in solution femtomol/kg	SF6, pptv corr. for excess air	Piston-flow model recharge year			
				CFC-11	CFC-12	CFC-113	CFC-11	CFC-12	CFC-113				CFC-11	CFC-12	CFC-113	SF6
Poe Springs	09/24/03	21.3	40	4.81	261	0.0	7.9	107	0.0	3.0	1.060	2.75	1956.0	1976.5	1953.0	1991.5
Poe Springs	09/24/03	21.3	40	4.48	253	0.0	7.4	104	0.0	3.0	1.203	3.12	1955.5	1976.0	1953.0	1993.5
Poe Springs	12/18/03	22.0	50	3.90	225	0.0	6.2	89.6	0.0	2.7	1.149	3.11	1955.0	1975.0	1953.0	1993.5
Poe Springs	12/18/03	22.0	50	3.42	224	0.0	5.4	89.0	0.0	2.7	1.261	3.41	1954.5	1974.5	1953.0	1994.5
Poe Springs	04/21/04	21.7	50	7.37	272	0.0	11.9	110	0.0	3.0	0.940	2.44	1957.5	1977.0	1953.0	1990.0
Poe Springs	04/21/04	21.7	50	4.92	260	0.0	8.0	105	0.0	3.0	1.032	2.68	1956.0	1976.5	1953.0	1991.5
Hornsby Spring	09/25/04	21.4	40	44.8	1549	3.26	73.3	634	2.2	2.3	0.553	1.50	1968.0	Contam.	1965.5	1985.0
Hornsby Spring	09/25/04	21.4	40	48.7	1555	3.23	79.8	636	2.2	2.3	0.593	1.60	1968.5	Contam.	1965.5	1985.0
Hornsby Spring	09/25/04	21.4	40	9.91	1530	1.83	16.2	626	1.2	2.3			1959.5	Contam.	1961.5	
Hornsby Spring	12/17/03	20.0	50	48.7	1775	1.90	84.7	760	1.3	2.3	0.642	1.74	1968.5	Contam.	1962.0	1986.5
Hornsby Spring	12/17/03	20.0	50	32.7	1778	2.31	57.0	761	1.6	2.3	0.671	1.82	1966.0	Contam.	1963.5	1987.0
Hornsby Spring	12/17/03	20.0	50	41.9	1782	1.94	72.9	763	1.4	2.3			1967.5	Contam.	1962.0	
Hornsby Spring	04/20/04	20.0	50	45.7	1535	3.11	79.6	657	2.2	2.3	0.551	1.49	1968.0	Contam.	1965.5	1985.0
Hornsby Spring	04/20/04	20.0	50	36.4	1377	2.53	63.3	590	1.8	2.3	0.558	1.51	1966.5	Contam.	1964.0	1985.0

Microbiological Indicators:

2004 Springs Study (Hornsby, Poe, and Manatee springs) - Microbiology Data

Dale W. Griffin and Mike A. Gray

Introduction

Microbiological sampling and viral tracer studies were conducted in synchronization with geochemical studies at Hornsby, Poe, and Manatee Springs. Standard and alternate microbial water quality indicators were used to screen water samples to determine if spring waters (Poe and Hornsby Springs) were being impacted by fecal sources. Source tracking assays were employed to differentiate between human (agricultural) and animal fecal contamination using human and bovine specific virus assays. All detection protocols included appropriate controls to account for false positive results. All sample collection and processing used sterile equipment and technique. The following procedures were used for each type of assay:

1. Microbial standard and alternate indicators.
    a) Fecal coliforms and *E.coli*.(APHA 1998).
    b) Enterococci.(APHA 1998).
    c) *Clostridium perfringens(Armon 1988)*.

2. Polymerase Chain Reaction (PCR) and Reverse-Transcriptase PCR for the detection of human (enteroviruses, adenoviruses, Hepatitis A viruses) and bovine specific viruses (enteroviruses) (Griffin 1999, Katayama 2002, Pina 1998b).

Methods/Results

Water samples from each spring were collected using sterile 9 liter carboys. After collection samples were placed on ice and transported to the USGS laboratory for processing. For each bacterial indicator analyzed (enterococci, *Clostridium perfringens*, and fecal coliforms), 500 mL of water was screened in duplicate using membrane filtration. *Escherichia coli* analysis was performed by transferring fecal coliform membranes to EC-MUG medium following fecal coliform incubation and enumeration. The indicator data is listed in Tables 1, 2, and 3. The results are reported as the average number (between the duplicates) in 100 mL of analyzed water.

Viral analysis was performed on the remaining aliquots of water using a modified method as reported by Katayama et al. (2002). In short, the remaining aliquots of water from each spring were filtered through a charged membrane filter. Aliquots varied from 1.5 liters to 6 liters depending on the particle load of the water. Viruses adsorbed onto the charged membrane were then eluted from the filter surface and further concentrated and purified to a final volume below 1 mL. Aliquots of these concentrates were then screened for the presence of the specific groups of viruses using RT-PCR/PCR. Any positive results were cloned and sequenced to confirm their identification. The virus data is listed in Tables 1, 2, and 3 and reported as positive (+) or negative (-). Equivalent volume assayed (the volume of concentrate screened, converted to the equivalent starting volume in the original unconcentrated sample) is reported in the first virus column (enteroviruses).

The bacteria indicator data for the 9-25-03 sample date (Table 1) demonstrated good water quality in all of the springs as it pertains to existing drinking water indicator standards; no viruses were detected. Bacteria indicator data for 12-17-03 (Table 2) was similar for Hornsby and Poe springs (they met drinking water standards) but elevated numbers of enterococci and fecal coliforms were observed at Manatee springs. Again, no viruses were detected at any of the springs on this sample date. Bacteria indicator for 4-24-04 (Table 3) demonstrates elevated fecal coliform concentrations in all springs relative to the other sample dates. Human enterovirus sequences were also detected at all springs on this date. The positive virus results were detected using gel electrophoresis and dot blot/genetic probes. Virus sequences were further confirmed by genetic cloning, DNA sequencing and GenBank Blast (this is a DNA data base that allows one to submit DNA sequences for comparison of existing sequences for microbial/viral identification). The PCR viral signal was highest in the Manatee springs sample followed by Poe and Hornsby springs respectively. This observed high to low PCR signal can correspond to high to low virus concentrations or it can indicate that sample components may have interfered with efficient amplification of target sequences. Virus sequences obtained from Manatee (100% sequence homology, 195bp/195bp) and Poe springs (98%, 152bp/154bp) samples indicated that these viruses were related to a vaccine strain of polio virus (GenBank # AF538842.1). We are still screening clones for the Hornsby spring sample to identify those sequences.

Conclusion

While the sample set is small (three sites, three dates) the data indicates that Hornsby and Poe spring water quality was consistently superior to that observed at Manatee springs. Hornsby and Poe spring water quality met microbial drinking water standards on two of the three sample dates and all the springs met recreational water quality standards on all sample dates.

Currently there are no viral water quality standards. In this study we utilized virus assays in order to determine if human wastewater (originating from septic systems, etc.) may be impacting each springs regional groundwater sources (via surface recharge). The virus data indicates that all of the springs in this study may be susceptible to fecal pollution of human origin at various times of the year. It is important to understand that RT-PCR detection of human enteroviruses does not differentiate between live/infectious and inactivated/dead viruses. It has been well documented that inactivated virus genomes may persist in the environment long after live viruses can no longer be detected (weeks to several months)(Jarrell 2001). It is interesting to note that viruses were detected on the date which corresponded with the presence of the highest numbers of bacterial indicators for each spring.

In conclusion, the microbiological data warrants the need for a more comprehensive long term study in order to address temporal and environmental issues that may influence regional ground water quality.

Table 1. Microbiology data (spring sample data for 9-25-03)

Spring	pH/Temperature-°C/EC-µS/TDS-ppm	Indicator Data – Colony Forming Units/100ml (duplicate average)				Virus Polymerase Chain Reaction data (Equivalent volume assayed)			
		Enterococci	*Clostridium perfringens*	Fecal coliforms	*E.coli*	Enteroviruses	Hepatitis A viruses	Adenoviruses	Cattle enteroviruses
Hornsby	6.9/22.8//298/150	0	0	0.2	0.1	- (0.04L)	-	-	-
Poe	7.4/-/287/143	0.1	0	0	0	- (0.5L)	-	-	-
Manatee	7.3/-/329/164	0.2	0	0.2	0.1	- (0.1L)	-	-	-

Table 2. Microbiology data (spring sample data for 12-17-03)

Spring	pH/Temperature-°C /EC-µS/TDS-ppm	Indicator Data – Colony Forming Units/100ml (duplicate average)				Virus Polymerase Chain Reaction data			
		Enterococci	*Clostridium perfringens*	Fecal coliforms	*E.coli*	Enteroviruses	Hepatitis A viruses	Adenoviruses	Cattle enteroviruses
Hornsby	7.1/22.0/271/131	0.2	0	0	0	- (0.3L)	-	-	-
Poe	7.1/22.1/266/133	0	0	0	0	- (0.3L)	-	-	-
Manatee	7.0/22.0/311/155	2.0	0	4.7	4.7	- (0.3L)	-	-	-

Table 3. Microbiology data (spring sample data for 4-20-04)

Spring	pH/Temperature-°C /EC-µS/TDS-ppm	Indicator Data – Colony Forming Units/100ml (duplicate average)				Virus Polymerase Chain Reaction data			
		Enterococci	*Clostridium perfringens*	Fecal coliforms	*E.coli*	Enteroviruses	Hepatitis A viruses	Adenoviruses	Cattle enteroviruses
Hornsby	7.2/22.5/276/139	1.2	0	3.0	3.0	+ (0.5L)	-	-	-
Poe	7.2/22.5/273/137	0.1	0	4.5	4.5	++ (0.5L)	-	-	-
Manatee	7.1/22.5/320/158	0.4	0	14.7	14.7	+++(0.5L)	-	-	-

+ = light signal via genetic probe/dot blot
++ = medium signal via genetic probe/dot blot
+++ = heavy signal via genetic probe/dot blot

Multiple Stable Isotope Analyses of Aquatic Biota

Ecological impact of nitrate contamination on Hornsby and Poe Springs

Lia C. Chasar

In aquatic ecosystems stable isotopes of carbon and nitrogen are useful as indicators of overall trophic status and as natural tracers of the transport and transformation of nutrients. The isotopic signatures of particulate organic matter, algae, and consumers (invertebrates and fishes) reflect the isotopic composition of available inorganic carbon and nutrients as well as the isotopic fractionation that occurs during uptake and assimilation (Goerike et al., 1994). Although diurnal and seasonal variability of elemental composition and isotopic ratios in sediments and biota may be relatively large (McCutcheon et al., 2003; Cloern et al., 2002), inputs of sewage and fertilizers to watersheds have been demonstrated to influence $\delta^{15}N$ values significantly enough to distinguish source and scale of such anthropogenic disturbances (Lake et al., 2001; Fry, 1999; Tucker et al., 1999; Cabana and Rassmussen, 1996; Mako and Ostrom, 1994). The comparative value of stable isotope ratios and elemental composition is derived from the range of temporal and spatial integration by algae, submerged/emergent aquatic vegetation (SAV/EAV), benthic macroinvertebrates and fish. This integration occurs on timescales commensurate with nutrient cycling and tissue turnover for each of these groups ranging from daily (microalgae) to weekly (SAV/EAV)to seasonally (macroinvertebrates and small forage fish) to interannually (larger forage fish and apex predators such as largemouth bass). This integrated signature contributes to an understanding of the sources of eutrophication, as well as the temporal and spatial extent of ecosystem response.

Methods

<u>Sample Collection and Analysis</u>

Biological samples were collected on the same days as geochemical samples, and comprised periphyton, epibenthic invertebrates, and forage fish. Samples were collected from pool margins, pool interior, margins of spring runs, and across spring run channels; because of the short spring run at Poe Springs samples were also collected from adjacent river margins both upstream and downstream. Spring pools and runs at both sites were heavily loaded in with thick mats of filamentous benthic algae; algal biomass was lower in December 2004, and mats appeared to be degrading at both sites but we were able to collect sufficient sample on all dates. We were able to collect Gammarid amphipods (primarily *Hyalella azteca*, with occasional *Gammarus* sp.) and mosquitofish at both sites and all seasons. Other consumer species were collected, but amphipods and mosquitofish were the only species represented at both sites and on all sampling events.

Three replicates were collected for each sample type; all samples were composited in the field. For composites of invertebrates the target for each replicate was 30-120 individuals depending on the mass of tissue; for composites of forage fish the target was 6-12individuals per replicate. Samples were placed on ice and transported to the Florida Integrated Sciences Center-Tallahassee, US Geological Survey where algae were rinsed with free of sediment and debris with de-ionized water; invertebrates and fish were identified to lowest taxon possible; and all samples were dried to constant weight in a vacuum oven at 50°C and ground to a fine powder with a Crescent Dental Wig-l-Bug (tissue amalgamator). All samples were analyzed for elemental composition (%C, %N) and stable isotopes (δ^{13}C, δ^{15}N) by Isotope Services in Los Alamos, New Mexico.

Results

A comparison of mean values for periphyton and consumers (amphipods and mosquito fish) is provided in Table 1 and all raw data are provided in Appendix 3.

The δ^{13}C signatures of mixed filamentous algae suggest that they were not a significant direct source of food to invertebrates and forage fish at either site - carbon only fractionates approximately 1 per mil per trophic level, and all invertebrates were enriched approximately 10 per mil relative to periphyton at both sites (Figure 1). This is not surprising,; while filamentous algae may support consumers as habitat or as a provide substrate for other periphyton (diatoms, microzooplankton) which may be grazed, these algal mats have not been demonstrated as a major diet item for invertebrates or small fishes in other systems.

Algae in Hornsby Spring were slightly enriched (more positive) relative to algae in Poe Springs (by 2 per mil in the pools, and by approximately 0.5 per mil in spring runs); water from spring vents at these springs showed an opposite trend in December 2003 and April 2004 (Poe enriched by approximately 5 per mil relative to Hornsby). Carbon and nitrogen signatures in algae may differ because of differences in species assemblages; similar assemblages may vary daily or even hourly in response to physicochemical factors (including flow, temperature, light availability). Analysis of variance (ANOVA) and pairwise comparisons (Tukey's test) of invertebrates and fish indicate that these biota are consistently enriched in ^{15}N at Poe Springs relative to Hornsby Spring. These data reflect a seasonally integrated signature and indicate that during our study the production in Poe Springs was primarily driven by a nitrogen source that was more enriched in ^{15}N than Hornsby Spring. As described in the preceeding section on geochemistry, possible contributor factors to consistently and significantly enriched ^{15}N signatures in water and biota include animal waste or denitrification. At both springs, biota in spring runs was significantly depleted relative to spring pools. At Poe Springs all biota were progressively depleted, i.e. δ^{15}N in spring pool > spring run > river proper.

At both sites, δ^{13}C and δ^{15}N of invertebrates and fish are more enriched in April than in December; this may reflect changes in loading but also probably reflects seasonal differences in temperature and photosynthetically active radiation (PAR); temperature affects dissolution, and temperature and PAR both affect metabolic processes.

Figure 1. Stable isotope (δ^{15}N vs δ^{13}C) crossplots for algae and invertebrates. Samples collected from river margins are noted as "DS" for downstream of outflow and "US" for upstream of outflow.

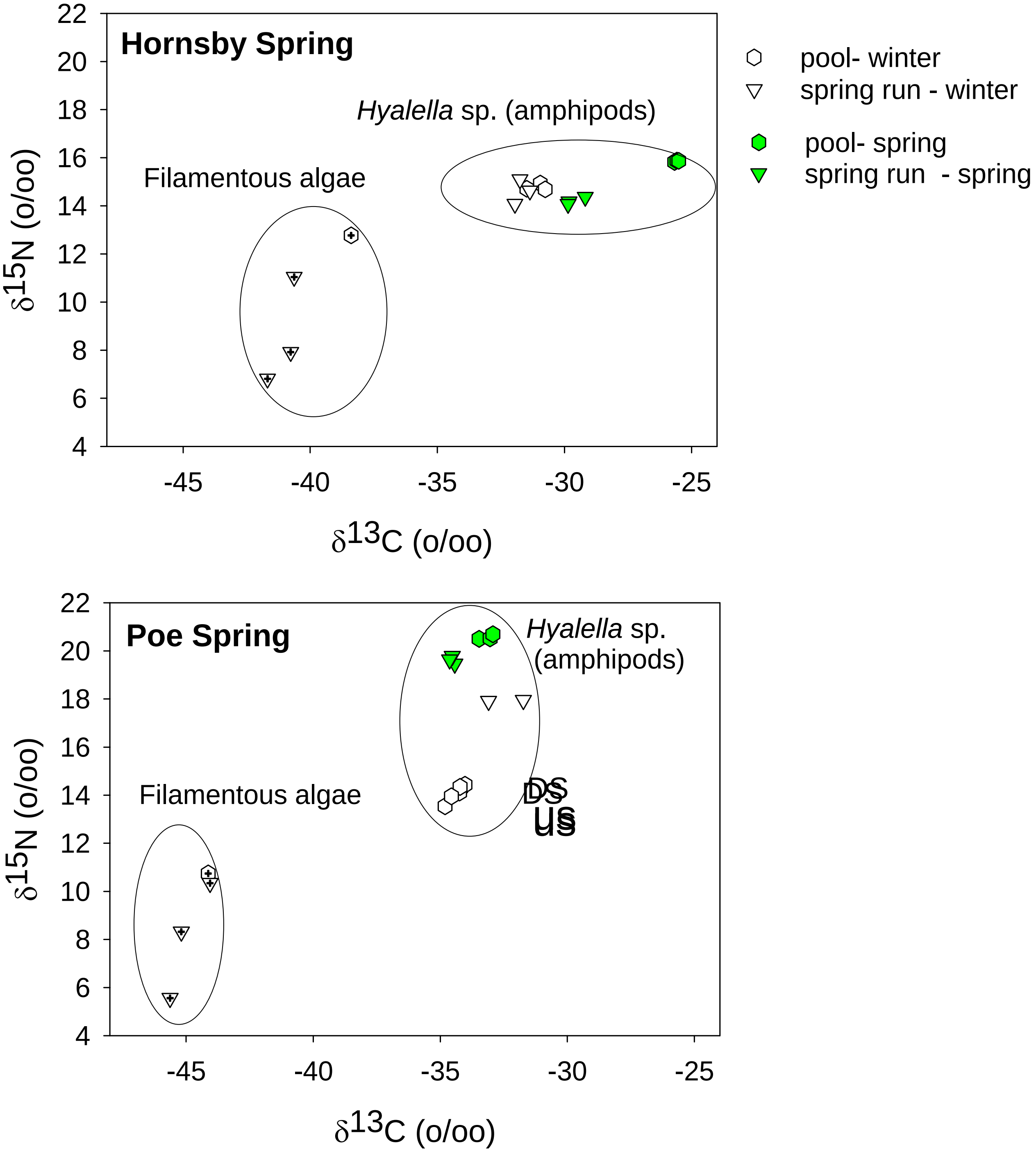

Table 1. Summary stable isotope data for biota at both Hornsby and Poe Springs. Values for $\delta^{13}C$ and $\delta^{15}N$ are expressed in per mil (‰) and standard deviations are provided in parentheses. All samples were collected from pool margins and central pool for "spring pool", from both margins and stream channel for "outflow", and along river margins adjacent to outflow for "river, upstream" and "river, downstream". All algae were composited grab samples of mixed filamentous algae. Amphipods were mixed species, primarily *Hyalella azteca* and occasional *Gammarus* sp.; each sample was a composite of 30-120 individuals and two replicates. Eastern mosquitofish were identified as *Gambusia holbrooki*, with pooled samples of 6-12 with two replicates.

	Filamentous Algae		*Amphipods*				*Eastern Mosquitofish*			
	12/17/2003-12/18/2003		12/17/2003-12/18/2003		4/20/2004-4/21/2004		12/17/2003-12/18/2003		4/20/2004-4/21/2004	
	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
Hornsby Spring										
spring pool	-38.39	12.77	-31.06	14.78	-25.59	15.86	-30.37	15.94	-29.28	18.54
	(0.00)	(0.00)	(0.37)	(0.13)	(0.08)	(0.03)	(0.47)	(1.04)	(1.16)	(1.26)
outflow	-41.03	8.59	-31.69	14.60	-29.62	14.21	-31.27	14.60	-29.72	14.21
	(0.57)	(0.00)	(0.30)	(0.51)	(0.38)	(0.15)	(0.00)	(0.51)	(0.96)	(0.15)
Poe Springs										
spring pool	-44.13	10.74	-34.34	14.11	-33.15	20.57	-32.37	18.46	-31.66	23.61
	(0.00)	(0.00)	(0.29)	(0.33)	(0.29)	(0.10)	(1.18)	(2.90)	(1.42)	(2.54)
outflow	-44.96	8.07	-32.49	17.82	-34.53	19.62	-34.11	19.20	-32.01	22.44
	(0.81)	(0.00)	(0.70)	(0.19)	(0.10)	(0.16)	(0.00)	(0.00)	(0.05)	(0.52)
river, upstream	-42.18	6.48			-30.15	13.06	-32.28	14.22		
	(0.00)	(0.00)			(0.01)	90.18)	(0.00)	(0.00)		
river, downstream	-42.40	6.03			-30.88	14.18	-32.28			
	(0.00)	(0.00)			(0.15)	(0.15)	(0.00)			

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Appendix 1. List of organic wastewater compounds analyzed by the U.S. Geological Survey National Water Quality Laboratory with method reporting limits (Zaugg and others, 2002).

Compound name	**Method reporting limit, ug/L**
Fragrances and Flavorants:	
Acetyl hexamethyl tetrahydronaphthalene (AHTN)	0.5
Hexadydrohexamethylcyclopentabenzopyran (HHCB)	0.5
3-Methyl-1(H)-indole (Skatole)	1.0
Acetophenone	0.5
Camphor	0.5
Isoborneol	0.5
Isoquinoline	0.5
Menthol	0.5
Flame Retardants:	
Tris(2-dichloroethyl)phosphate	0.5
Tris(2-chloroethyl)phosphate	0.5
Tributyl phosphate	0.5
Antioxidants:	
5-Methyl-1H-benzotriazole	2.0
3-tert-Butyl-4-hydroxy anisole (BHA)	5.0
Fuel-related compounds:	
1-Methylnaphthalene	0.5
2,6-Dimethylnaphthalene	0.5
2-Methylnaphthalene	0.5
Isopropylbenzene (cumene)	0.5
Detergent metabolites:	
p-Cumylphenol	1.0
p-n-Octylphenol	1.0
p-Nonylphenol diethoxylate (NPEO2)	5.0
p-Octylphenol diethoxylate (OPEO2)	1.0
p-Octylphenol monoethoxylate (OPEO1)	1.0
p-tert-octylphenol	1.0
p-Nonylphenol (total NP)	5.0
Plasticizers:	
Bisphenol A	1.0
Tris(2-butoxyethyl) phosphate	0.5
Triphenyl phosphate	0.5
Disinfectants:	
Phenol	0.5
Triclosan	1.0
Solvents and preservatives:	
Isophorone	0.5

Appendix 1. (cont'd) List of human wastewater compounds analyzed by the U.S. Geological Survey National Water Quality Laboratory with method reporting limits (Zaugg and others, 2002).

Compound name	**Method reporting limit, ug/L**
Tetrachloroethylene	0.5
p-Cresol	1.0
Pentachlorophenol	2.0
PAHs:	
Anthracene	0.5
Benzo[a]pyrene	0.5
Fluoranthene	0.5
Naphthalene	0.5
Phenanthrene	0.5
Pyrene	0.5
Pesticides:	
Bromacil	0.5
Carbaryl	1.0
Carbazole	0.5
Chlorpyrifos	0.5
Diazinon	0.5
Dichlorvos	1.0
d-Limonene	0.5
Indole	0.5
Metalaxyl	0.5
Metalachlor	0.5
N,N-diethyl-meta-toluamide (DEET)	0.5
Prometon	0.5
Plant and animal steroids:	
3-beta-Coprostanol	2.0
beta-Sitosterol	2.0
beta-Stigmastanol	2.0
Cholesterol	2.0
Others:	
Anthraquinone (manufacturing)	0.5
1,4-Dichlorobenzene (deodorizer)	0.5
Benzophenone (fixative)	0.5
Bromoform (disinfection byproduct)	0.5
Caffeine (stimulant)	0.5
Cotinine (nicotine metabolite)	1.0
Methyl salicylate (liniment)	0.5
Triethyl citrate (ethyl citrate) (cosmetics)	0.5

Appendix 2. Pesticides and selected degradate compounds analyzed at the U.S. Geological Survey National Water Quality Laboratory. Initial method detection limits (irl) and method reporting levels (mrl) are reported below in micrograms per liter. Method detection limits have not been determined for some analytes (ND).

1-Napthol		0.0882	ug/L	irl
2-Chloro-2,6-diethylacetanilide		0.005	ug/L	irl
2-Ethyl-6-methylaniline		0.0045	ug/L	irl
3,4-Dichloroaniline		0.0045	ug/L	irl
4-Chloro-2-methylphenol		0.0057	ug/L	irl
Acetochlor		0.006	ug/L	lrl
Alachlor		0.005	ug/L	lrl
2,6-Diethylaniline		0.006	ug/L	lrl
Atrazine		0.007	ug/L	lrl
Azinphos-methyl		0.05	ug/L	lrl
Azinphos-methyl-oxon		0.0700	ug/L	irl
Benfluralin		0.010	ug/L	lrl
Carbaryl		0.041	ug/L	lrl
Chlorpyrifos		0.005	ug/L	lrl
Chlorpyrofos, oxygen analog		0.0562	ug/L	irl
cis-Permethrin		0.006	ug/L	lrl
Cyfluthrin		0.008	ug/L	irl
Cypermethrin		0.0086	ug/L	irl
Dacthal		0.003	ug/L	lrl
2-Chloro-4-isopropylamino-6-amino-s-triazine		0.006	ug/L	lrl
Diazinon		0.005	ug/L	lrl
Diazinon, oxygen analog		0.006		irl
Diazinon-d10			pct	
Dichlorvos		0.0118	ug/L	irl
Dicrotophos		0.0843	ug/L	irl
Dieldrin		0.009	ug/L	lrl
Dimethoate		0.0061	ug/L	irl
Ethion		0.004	ug/L	irl
Ethion monoxon		0.0020	ug/L	irl
Fenamiphos		0.029	ug/L	irl
Fenamiphos sulfone		0.0491	ug/L	irl
Fenamiphos sulfoxide		0.0387	ug/L	irl
Desulfinylfipronil amide		0.029	ug/L	lrl
Fipronil sulfide		0.013	ug/L	lrl
Fipronil sulfone		0.024	ug/L	lrl
Desulfinylfipronil		0.012	ug/L	lrl
Fipronil		0.016	ug/L	lrl

Appendix 2. (cont'd) Pesticides and selected degradate compounds analyzed at the U.S. Geological Survey National Water Quality Laboratory. Initial method detection limits (irl) and method reporting levels (mrl) are reported below in micrograms per liter. Method detection limits have not been determined for some analytes (ND).

Fonofos		0.003	ug/L	lrl
Fonofos, oxygen analog		0.0029	ug/L	irl
alpha-HCH-d6			pct	
Hexazinone		0.0129	ug/L	irl
Iprodione		0.387	ug/L	irl
Isofenphos		0.0034	ug/L	irl
Malaoxon		0.0298	ug/L	irl
Malathion		0.027	ug/L	lrl
Metalaxyl		0.0051	ug/L	irl
Methidathion		0.0058	ug/L	irl
Parathion-methyl		0.015	ug/L	lrl
Metolachlor		0.013	ug/L	lrl
Metribuzin		0.006	ug/L	lrl
Myclobutanil		0.008	ug/L	irl
Paraoxon-methyl		0.0299	ug/L	irl
Pendimethalin		0.022	ug/L	lrl
Phorate		0.011	ug/L	lrl
Phorate oxygen analog		0.1048	ug/L	irl
Phosmet		0.0079	ug/L	irl
Phosmet oxon		0.0511	ug/L	irl
Prometon		0.005	ug/L	lrl
Prometryn		0.0054	ug/L	irl
Propyzamide		0.004	ug/L	lrl
Sample volume			mL	
Set number			no.	
Simazine		0.005	ug/L	lrl
Tebuthiuron		0.016	ug/L	lrl
Terbufos		0.017	ug/L	lrl
Terbufos oxygen analog sulfone		0.0676	ug/L	irl
Terbuthylazine		0.0102	ug/L	irl
Trifluralin		0.009	ug/L	lrl

Appendix 3. Stable Isotope data for all samples collected from Hornsby and Poe Springs September 2003-April 2004.

Sample ID	Site	Location	Collection Date	Description	Taxa	n	δ^{13}C (‰)	δ^{15}N (‰)
AL 20	Hornsby Spring	pool	12/17/2003	ephemenopterans	*Caenis sp.*	60	-33.38	14.39
AL 17	Hornsby Spring	pool	12/17/2003	amphipods	*Hyalella azteca*	30	-30.95	14.93
AL 18	Hornsby Spring	pool	12/17/2003	amphipods	*Hyalella azteca*	30	-31.48	14.71
AL 19	Hornsby Spring	pool	12/17/2003	amphipods	*Hyalella azteca*	30	-30.76	14.69
AL 115	Hornsby Spring	pool	4/20/2004	amphipods	*Hyalella azteca*	90	-25.67	15.82
AL 116	Hornsby Spring	pool	4/20/2004	amphipods	*Hyalella azteca*	90	-25.58	15.88
AL 117	Hornsby Spring	pool	4/20/2004	amphipods	*Hyalella azteca*	90	-25.51	15.87
AL 74	Hornsby Spring	pool	9/24/2003	mixed filamentous	unknown		-39.31	10.95
AL 38	Hornsby Spring	pool	12/17/2003	crayfish 5cm	unknown	3	-27.47	12.99
AL 27	Hornsby Spring	pool	12/17/2003	mixed filamentous	unknown		-38.39	12.77
AL168	Hornsby Spring	pool	12/17/2003	mosquito fish	*Gambusia holbrooki*	8	-30.5	16.76
AL169	Hornsby Spring	pool	12/17/2003	mosquito fish	*Gambusia holbrooki*	8	-30.76	16.3
AL170	Hornsby Spring	pool	12/17/2003	mosquito fish	*Gambusia holbrooki*	5	-29.85	14.77
AL162	Hornsby Spring	pool	4/20/2004	mosquito fish	*Gambusia holbrooki*	9	-29.39	18.47
AL163	Hornsby Spring	pool	4/20/2004	mosquito fish	*Gambusia holbrooki*	9	-29.16	18.61
AL 123	Hornsby Spring	outflow	4/20/2004	isopods	*Caecidotea sp.*	60	-39.76	15.15
AL 11	Hornsby Spring	outflow	12/17/2003	ephemenopterans	*Caenis sp.*	90	-33.81	14.82
AL 121	Hornsby Spring	outflow	4/20/2004	ephemenopterans	*Caenis sp.*	40	-31.06	13.85
AL 122	Hornsby Spring	outflow	4/20/2004	ephemenopterans	*Caenis sp.*	40	-30.88	13.82
AL 42	Hornsby Spring	outflow	12/17/2003	mosquito fish	*Gambusia holbrooki*	8	-30.6	16.4
AL 43	Hornsby Spring	outflow	12/17/2003	mosquito fish	*Gambusia holbrooki*	8	-31.27	16.36
AL166	Hornsby Spring	outflow	4/20/2004	mosquito fish	*Gambusia holbrooki*	12	-29.83	17.8
AL167	Hornsby Spring	outflow	4/20/2004	mosquito fish	*Gambusia holbrooki*	12	-29.6	18.28
AL 10	Hornsby Spring	outflow	12/17/2003	amphipods	*Hyalella azteca*	30	-31.36	14.63
AL 8	Hornsby Spring	outflow	12/17/2003	amphipods	*Hyalella azteca*	30	-31.75	15.1
AL 9	Hornsby Spring	outflow	12/17/2003	amphipods	*Hyalella azteca*	30	-31.95	14.08
AL 118	Hornsby Spring	outflow	4/20/2004	amphipods	*Hyalella azteca*	60	-29.82	14.18
AL 119	Hornsby Spring	outflow	4/20/2004	amphipods	*Hyalella azteca*	60	-29.86	14.07
AL 120	Hornsby Spring	outflow	4/20/2004	amphipods	*Hyalella azteca*	60	-29.18	14.37
AL 75	Hornsby Spring	outflow	9/24/2003	mixed filamentous	unknown		-39.37	12.55
AL 16	Hornsby Spring	outflow	12/17/2003	mixed blue-green	unknown		-32.19	15.88

Appendix 3. (cont’d) Stable Isotope data for all samples collected from Hornsby and Poe Springs September 2003-April 2004.

AL 15	Hornsby Spring	outflow	12/17/2003	mixed filamentous	unknown		-41.68	6.81
AL 25	Hornsby Spring	outflow	12/17/2003	mixed filamentous	unknown		-40.63	11.04
AL 23	Hornsby Spring	outflow	12/17/2003	mixed filamentous	unknown		-40.77	7.92
AL 39	Hornsby Spring	outflow	12/17/2003	crayfish 4-6cm	unknown	4	-30.93	14.21
AL 40	Hornsby Spring	outflow	12/17/2003	crayfish 2-3cm	unknown	8	-31.6	15.82
AL 151	Hornsby Spring	outflow	4/20/2004	crayfish 6cm	unknown	3	-30.07	15.63
AL 152	Hornsby Spring	outflow	4/20/2004	crayfish2-3cm	unknown	6	-29.55	16.24
AL 29	Poe Spring	pool	12/18/2003	isopods	*Caecidotea sp.*	60	-33.95	13.39
AL 5	Poe Spring	pool	12/18/2003	isopods	*Caecidotea sp.*	60	-31.36	14.63
AL 107	Poe Spring	pool	4/21/2004	isopods	*Caecidotea sp.*	30	-33.1	18.14
AL 51	Poe Spring	pool	12/18/2003	mosquito fish	*Gambusia holbrooki*	6	-32.89	16.85
AL 52	Poe Spring	pool	12/18/2003	mosquito fish	*Gambusia holbrooki*	9	-31.84	20.06
AL 2	Poe Spring	pool	12/18/2003	amphipods	*Hyalella azteca*	60	-34.2	14.28
AL 3	Poe Spring	pool	12/18/2003	amphipods	*Hyalella azteca*	60	-34.03	14.43
AL 32	Poe Spring	pool	12/18/2003	amphipods	*Hyalella azteca*	40	-34.22	14.34
AL 33	Poe Spring	pool	12/18/2003	amphipods	*Hyalella azteca*	40	-34.24	14.11
AL 34	Poe Spring	pool	12/18/2003	amphipods	*Hyalella azteca*	40	-34.81	13.54
AL 4	Poe Spring	pool	12/18/2003	amphipods	*Hyalella azteca*	60	-34.56	13.95
AL 108	Poe Spring	pool	4/21/2004	amphipods	*Hyalella azteca*	100	-33.47	20.5
AL 109	Poe Spring	pool	4/21/2004	amphipods	*Hyalella azteca*	100	-33.04	20.52
AL 110	Poe Spring	pool	4/21/2004	amphipods	*Hyalella azteca*	100	-32.93	20.69
AL 50	Poe Spring	pool	12/18/2003	crayfish 2-4cm	*Procambarus spiculifer*	4	-33.57	13.48
AL 144	Poe Spring	pool	4/21/2004	crayfish 5-6cm	*Procambarus spiculifer*	2	-29.9	19.88
AL 76	Poe Spring	pool	9/24/2003	mixed filamentous	unknown		-48.14	1.92
AL 64	Poe Spring	pool	12/18/2003	mixed filamentous	unknown		-44.13	10.74
AL164	Poe Spring	pool	4/21/2004	mosquito fish	*Gambusia holbrooki*	9	-31.55	23.58
AL165	Poe Spring	pool	4/21/2004	mosquito fish	*Gambusia holbrooki*	12	-31.77	23.63
AL 56	Poe Spring	outflow	12/18/2003	isopods	*Caecidotea sp.*	90	-31.47	15.82
AL 101	Poe Spring	outflow	4/21/2004	isopods	*Caecidotea sp.*	60	-33.62	19.59
AL 53	Poe Spring	outflow	12/18/2003	mosquito fish	*Gambusia holbrooki*	8	-34.11	19.2
AL160	Poe Spring	outflow	4/21/2004	mosquito fish	*Gambusia holbrooki*	12	-31.97	22.07
AL161	Poe Spring	outflow	4/21/2004	mosquito fish	*Gambusia holbrooki*	10	-32.04	22.8
AL 65	Poe Spring	outflow	9/24/2003	amphipods	*Hyalella azteca*	30	-34.19	17.67

Appendix 3. (cont'd) Stable Isotope data for all samples collected from Hornsby and Poe Springs September 2003-April 2004.

AL 66	Poe Spring	outflow	9/24/2003	amphipods	*Hyalella azteca*	30	-34.07	18.24
AL 61	Poe Spring	outflow	12/18/2003	amphipods	*Hyalella azteca*	60	-32.63	17.61
AL 62	Poe Spring	outflow	12/18/2003	amphipods	*Hyalella azteca*	60	-33.1	17.91
AL 63	Poe Spring	outflow	12/18/2003	amphipods	*Hyalella azteca*	60	-31.73	17.95
AL 104	Poe Spring	outflow	4/21/2004	amphipods	*Hyalella azteca*	120	-34.43	19.46
AL 105	Poe Spring	outflow	4/21/2004	amphipods	*Hyalella azteca*	120	-34.53	19.78
AL 106	Poe Spring	outflow	4/21/2004	amphipods	*Hyalella azteca*	120	-34.63	19.63
AL 49	Poe Spring	outflow	12/18/2003	crayfish	*Procambarus spiculifer*	5	-32.93	20.98
AL 54	Poe Spring	outflow	12/18/2003	crayfish 4-5cm	*Procambarus spiculifer*	5	-35.56	16.37
AL 55	Poe Spring	outflow	12/18/2003	crayfish 3 cm	*Procambarus spiculifer*	3	-32.12	15.78
AL 133	Poe Spring	outflow	4/21/2004	crayfish 2cm	*Procambarus spiculifer*	12	-33.79	19.69
AL 134	Poe Spring	outflow	4/21/2004	crayfish 2cm	*Procambarus spiculifer*	12	-33.51	19.7
AL 77	Poe Spring	outflow	9/24/2003	mixed filamentous	unknown		-44.15	5.3
AL 71	Poe Spring	outflow	12/18/2003	mixed filamentous	unknown		-45.63	5.56
AL 70	Poe Spring	outflow	12/18/2003	mixed filamentous	unknown		-45.19	8.32
AL 69	Poe Spring	outflow	12/18/2003	mixed filamentous	unknown		-44.06	10.34
AL 44	Santa Fe River		12/18/2003	mosquito fish	*Gambusia holbrooki*	8	-32.28	14.22
AL 45	Santa Fe River		12/18/2003	crayfish 2-5cm	*Procambarus spiculifer*	4	-29.13	9.39
AL 113	Santa Fe River	downstream	4/21/2004	amphipods	*Hyalella azteca*	60	-30.77	14.28
AL 114	Santa Fe River	downstream	4/21/2004	amphipods	*Hyalella azteca*	60	-30.98	14.07
AL 128	Santa Fe River	downstream	4/21/2004	grass shrimp	*Palaemonetes paludosis*	12	-32.09	16.15
AL 129	Santa Fe River	downstream	4/21/2004	grass shrimp	*Palaemonetes paludosis*	12	-31.77	16.19
AL 132	Santa Fe River	downstream	4/21/2004	grass shrimp	*Palaemonetes paludosis*	12	-31.62	16.29
AL 131	Santa Fe River	downstream	4/21/2004	crayfish 4-5cm	*Procambarus spiculifer*	3	-32.56	16.59
AL 79	Santa Fe River	downstream	9/24/2003	mixed filamentous	unknown		-41.7	6.15
AL 73	Santa Fe River	downstream	12/18/2003	mixed filamentous	unknown		-42.4	6.03
AL 111	Santa Fe River	upstream	4/21/2004	amphipods	*Hyalella azteca*	60	-30.5	13.19
AL 112	Santa Fe River	upstream	4/21/2004	amphipods	*Hyalella azteca*	60	-30.51	12.93
AL 127	Santa Fe River	upstream	4/21/2004	grass shrimp	*Palaemonetes paludosis*	12	-29.01	13.91
AL 126	Santa Fe River	upstream	4/20/2004	crayfish 2-4cm	*Procambarus spiculifer*	5	-41.03	10.85
AL 78	Santa Fe River	upstream	9/24/2003	mixed filamentous	unknown		-37.6	8.36
AL 80	Santa Fe River	upstream	9/24/2003	mixed filamentous	unknown		-27.87	6.48
AL 72	Santa Fe River	upstream	12/18/2003	mixed filamentous	unknown		-42.18	6.48

Appendix 4. Organic wastewater compounds and pesticides detected in samples of water from Hornsby and Poe Springs, September 2003-April 2004.

Concentrations are in micrograms per liter. M, Presence of material verified but not quantified; E, estimated value below method reporting limit but quantifiable.

Sample Date	Sample Time	Discharge, instantaneous, cubic feet per second	P04025 Hexazinone	P34462 Phenanthrene	P34466 Phenol	P34476 Tetrachloroethene	P39415 Metolachlor	P39632 Atrazine	P50305 Caffeine	P62056 2-Methylnaphthalene	P62065 Acetyl hexamethyl tetrahydro naphthalene	P62067 Benzophenone	P62081 Methyl salicylate	P62082 DEET	P62084 p-Cresol	P62085 4-Nonylphenol	P62089 Tributyl phosphate	P62092 Triphenyl phosphate
HORNSBY SPRING- 2321970																		
9/25/2003	10:00	102	E .002	M	E .2	< .5	M	< .007	< .5	< .5	< .5	E .1	< .5	2.5	< 1	< 5	< .5	< .5
12/17/2003	9:45	61	< .013	< .5	< .5	< .5	E .004	E .004	< .5	< .5	< .5	< .5	< .5	0.6	< 1	< 5	< .5	< .5
4/20/2004	8:15	41	< .013	M	E .3	M	< .013	E .002	< .5	< .5	M	M	< .5	E .4	< 1	M	E .1	E .1
POE SPRING- 02322140																		
9/24/2003	13:00	82	E .008	< .5	< .5	< .5	< .013	0.007	M	< .5	< .5	E .1	< .5	7.5	< 1	< 5	< .5	< .5
12/18/2003	10:15	55	< .013	< .5	< .5	< .5	< .013	E .005	< .5	< .5	< .5	< .5	< .5	E .2	< 1	< 5	< .5	< .5
4/21/2004	10:00	42	E .005	M	0.6	M	< .013	E .003	< .5	M	M	M	M	E .2	M	E 1	E .1	E .1
Major chemical usage			pesticide	polyaromatic hydrocarbon (PAH)	disinfectant	solvent	herbicide	herbicide	stimulant	fuel-related compound	fragrance and flavorant	fixative	liniment	insect repellent	solvent and preservative	detergent metabolite	flame retardant	plasticizer