SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 27, No. 12, pp. 2457–2468, 2008
© 2008 SETAC
Printed in the USA
0730-7268/08 $12.00 + .00

WASTEWATER-CONTAMINATED GROUNDWATER AS A SOURCE OF ENDOGENOUS HORMONES AND PHARMACEUTICALS TO SURFACE WATER ECOSYSTEMS

LAUREL J. STANDLEY,*† RUTHANN A. RUDEL,† CHRISTOPHER H. SWARTZ,‡ KATHLEEN R. ATTFIELD,† JEFF CHRISTIAN,§ MIKE ERICKSON,§ and JULIA G. BRODY†
†Silent Spring Institute, 29 Crafts Street, Suite 150, Newton, Massachusetts 02458, USA
‡Stockholm Environment Institute, 11 Curtis Avenue, Somerville, Massachusetts 02144, USA
§Columbia Analytical Services, 1317 South 13th Avenue, Kelso, Washington 98626, USA

(*Received* 21 *November* 2007; *Accepted* 11 *June* 2008)

Abstract—Increasing residential development in watershed recharge areas increases the likelihood of groundwater and surface water contamination by wastewater effluent, particularly where on-site sewage treatment is employed. This effluent contains a range of compounds including those that have been demonstrated to mimic or interfere with the function of natural hormones in aquatic organisms and humans. To explore whether groundwater contaminated by discharge from on-site septic systems affects water quality in surface water ecosystems, we measured steroidal hormones, pharmaceuticals, and other organic wastewater compounds (OWCs) in water collected from six aquifer-fed ponds in areas of higher and lower residential density on Cape Cod (Massachusetts, USA). We detected both a greater number and higher concentrations of OWCs in samples collected from ponds located in higher residential density areas. Most often detected were the steroidal hormones androstenedione, estrone, and progesterone and the pharmaceuticals carbamazepine, pentoxifylline, sulfamethoxazole, and trimethoprim. Of particular concern, estrogenic hormones were present at concentrations approaching those that induce physiological responses in fish. While a number of papers have reported on surface water contamination by OWCs from wastewater treatment plants, our results show that surface water ecosystems in unconfined aquifer settings are susceptible to contamination by estrogenic and other biologically active OWCs through recharge from aquifers contaminated by residential septic systems.

Keywords—Endocrine disruptors Pharmaceuticals Surface water On-site septic system Groundwater

INTRODUCTION

Expansion of residential development into watershed recharge areas increases the likelihood of contamination in not only surface water but also groundwater resources by wastewater effluent. This effluent contains a variety of organic wastewater compounds (OWCs), including excreted hormones and pharmaceuticals, detergent components, and disposed household and personal care products that are of concern because of their ability to interfere with the function of natural hormones in both aquatic organisms and humans [1–5]. Many of these compounds have already been widely documented in surface waters receiving discharge from wastewater treatment plants [6–11]. However, less attention has been paid to transport of OWCs to surface waters by wastewater-contaminated groundwater.

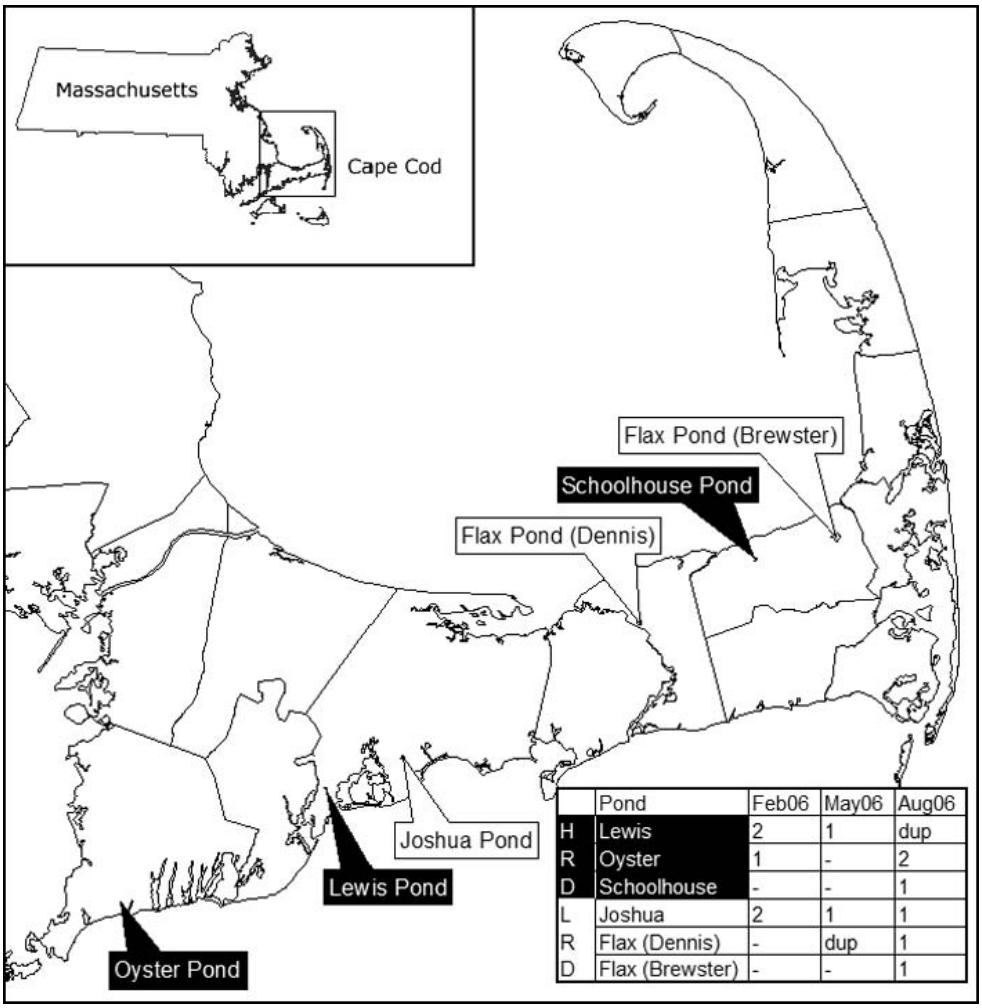
Recently, OWCs have been detected in groundwater in areas where on-site septic systems predominate, wastewater effluent is discharged onto land surfaces, or recharge from effluent-contaminated surface waters infiltrates through the hyporheic zone [10,12–17]. For example, on Cape Cod (Massachusetts, USA), where septic systems service more than 85% of commercial and residential development, traditional indicators such as nitrate show that groundwater quality has been declining for decades [18]. More recently, researchers also detected nonylphenol, bisphenol A, and the hormones β-estradiol and estrone in groundwater [12,15,16]. In particular, nonylphenol and other OWCs were detected in Cape Cod groundwater more than 5 m downgradient of a multi-family septic system [15] and 0.5 km downgradient from a wastewater infiltration bed [16]. Although the porous nature of Cape Cod sandy aquifers increases their vulnerability to contamination, this phenomenon is not unique to this area. For example, contamination of groundwater by septic system discharge containing OWCs has also been reported in other regions of the country including central Oregon [14], Montana [17,19], Nevada [13], and Nebraska [20], USA. Several studies have documented high concentrations of endocrine disrupting compounds and pharmaceuticals in effluent of on-site septic systems [15,17,21], increasing the likelihood that discharge from these systems will contaminate vulnerable aquifers, particularly where an anoxic core of the wastewater plume is maintained [15,22].

The introduction of septic system wastewater containing nutrients and OWCs into groundwater raises concerns not only regarding the quality of groundwater-derived drinking water but also impacts to surface water ecosystems that are primarily fed by groundwater. Eutrophication impacts are readily apparent, for example, in Washington State, where lakes surrounded by residences using septic systems experienced greater eutrophication than those in sewered areas with higher residential density [23]. Similarly, on Cape Cod, discharges of nutrients from on-site septic systems have been implicated as a primary factor in the eutrophication of hydraulically connected ponds [18,24]. The potential for OWC-contaminated groundwater to contaminate surface waters was recently doc-

* To whom correspondence may be addressed (standley@silentspring.org).

Presented at the 27th North American Society of Environmental Toxicology and Chemistry meeting, in Montreal, Quebec, Canada, November 5–9, 2006.

Published on the Web 7/10/2008.



	Pond	Feb06	May06	Aug06
H	Lewis	2	1	dup
R	Oyster	1	-	2
D	Schoolhouse	-	-	1
L	Joshua	2	1	1
R	Flax (Dennis)	-	dup	1
D	Flax (Brewster)	-	-	1

Fig. 1. Map showing location of study ponds on Cape Cod (Massachusetts, USA) (inset map showing location of Cape Cod in Massachusetts; inset table showing number of samples collected at three time points in 2006).

umented using seepage meters by Peeler et al. [25], who reported an influx of caffeine to Sarasota Bay (Florida, USA).

To investigate the extent of contamination of surface water ecosystems by wastewater-contaminated groundwater, we measured steroidal hormones, pharmaceuticals, and other OWCs in six aquifer-fed ponds on Cape Cod, Massachusetts, located in areas of higher and lower residential density. We sampled the ponds during three seasons to include an assessment of whether the large increase in population during the summer tourist season influenced contaminant burdens. While a number of papers have reported on surface water contamination from wastewater treatment plant effluent, the present study documents for the first time contamination of surface waters by endogenous hormones and pharmaceuticals from aquifers receiving discharge from on-site septic systems. This issue is of particular relevance for unconfined sand and gravel aquifers where long-range migration of contaminants in groundwater is likely [16,26].

MATERIALS AND METHODS

Site selection

On Cape Cod, groundwater flows through an unconfined stratified sand and gravel aquifer, with porewater velocities ranging from 0.15 to 0.3 m/d [27]. Cape Cod's kettle ponds were formed as depressions remaining in the terrain when glaciers retreated north about 12,000 years ago [26]. These ponds typically lack streams flowing into them and are thus fed primarily by groundwater [26].

We selected study ponds on Cape Cod using two criteria intended to ascertain likelihood of contamination by septic system discharge. First, we selected ponds with total nitrogen concentrations measured in 2001 that were higher (impacted) or lower (unimpacted) than the U.S. Environmental Protection Agency's (U.S. EPA) reference threshold (0.32 mg/L for Cape Cod [26,28]). We then determined proximity to residential density by examining aerial photographs (www.mass.gov/mgis/colororthos2005.htm, accessed March 11, 2008) and land use designations (www.mass.gov/mgis/lus.htm, accessed March 11, 2008) in the vicinity of these ponds to select those located in higher (houses on majority of perimeter) and lower (few, if any, houses on perimeter) residential density areas. We eliminated those located downgradient of other potential sources of OWCs, such as agricultural, commercial (e.g., pharmacies), and industrial areas. No ponds receiving wastewater treatment plant discharge were included in the present study. In all, we selected six ponds across Cape Cod (Fig. 1), with three located in higher residential density (HRD, black labels) areas and three in lower residential density (LRD, white labels) areas. The LRD ponds provided a benchmark for conditions of minimal contamination by wastewater.

Lewis (town of Barnstable, MA, USA), Oyster (town of Falmouth, MA, USA), and Schoolhouse (town of Brewster, MA, USA) ponds were the three HRD ponds selected for the present study. Lewis Pond is surrounded by low and medium density development along the upgradient and downgradient perimeters. Visibility was very low in this pond and the smell of wastewater was apparent during warmer months. We sampled this pond during all three seasons. The second HRD pond, Oyster Pond, is the only brackish pond in the study, receiving seawater through a causeway on its downgradient perimeter (www.opet.org). Residential development along the perimeter of Oyster Pond is primarily medium density (quarter to half acre lots), with the exception of a high-density senior condominium development to the north (upgradient) and a section without development located along the northeastern edge. No wastewater smell was apparent in this pond during sampling, which occurred in February and August 2006. Schoolhouse Pond was the third HRD pond sampled and is surrounded by low-density development (less than half acre lots). This pond was sampled only in August 2006 and water was a deep tea color.

Joshua Pond (town of Barnstable), Flax Pond (town of Dennis, MA, USA), and Flax Pond (town of Brewster) were the three LRD ponds selected. The two Flax Ponds will be distinguished by their town names. Joshua Pond was sampled during all three seasons and was devoid of residences along its perimeter except for the presence of a medium density neighborhood along its southwestern (downgradient) edge. Visibility was excellent and access for recreation included a beach located on the downgradient perimeter. Flax Pond (Dennis), sampled in May and August 2006, was bordered in part by a high-density development (less than quarter acre lots) along its western perimeter (lateral to flow). Also, we noted, and residents reported, frequent recreation by dogs in both Joshua and Flax (Dennis) ponds, indicating an additional source for selected OWCs, such as β-estradiol and veterinary medications. The third LRD pond, Flax Pond (Brewster), is located in Nickerson State Park (MA, USA). Although no residences are located along the perimeter, several septic systems are associated with camping areas near this pond (J. Peterson, Nickerson State Park Supervisor, personal communication). Flax Pond (Brewster) was sampled only in August 2006.

Sample collection

We collected water samples from ponds (Fig. 1, inset table) for analysis of organic and inorganic wastewater compounds. In February 2006, we sampled three ponds (LRD, Joshua Pond; HRD, Lewis and Oyster Ponds). We then collected samples in May 2006 from two of the ponds sampled in February (Joshua and Lewis ponds) and a second LRD pond (Flax Pond, Dennis) to provide a pretourist season sampling. Finally, we sampled all six ponds in late August 2006.

Pond water was collected in 1-L precleaned amber glass bottles (prespiked with 2.0 ml H_2SO_4 to inhibit microbial degradation [29]) for analysis of organic analytes. Water samples were also collected in polyethylene bottles for analysis of dissolved organic carbon (DOC [30]), nitrate, and boron, with nitric acid preservative (pH < 2) added to samples for boron analysis. Water was sampled from central locations in ponds at approximately half the maximum depth in ponds (estimated from the Cape Cod Pond and Lake Atlas [26] and by dropping a line to the bottom) using peristaltic pumps; all apparatus in contact with samples were constructed of glass, high purity silicone, or Teflon®. Samples were shipped with ice packs, stored in the laboratory at 4°C, and extracted within 14 d of sample collection.

A YSI 600 XL probe (YSI Life Sciences, Yellow Springs, OH, USA) was utilized to collect measures of temperature (°C), pH, dissolved oxygen (mg/L), and conductivity (μS/cm) at six points across the pond and at two depths to determine the vertical and horizontal heterogeneity in Lewis Pond in July 2007.

Analytical methods

Samples were analyzed for 29 organic and three nutrients or inorganic wastewater contaminants. Organic analytes included endogenous hormones, pharmaceuticals, and other wastewater contaminants (see Table 1). Nutrients and inorganic wastewater contaminants analyzed were (reporting limits in parentheses): nitrate-nitrogen (NO_3-N, 0.1 mg/L), boron (50 μg/L for February samples and 10 μg/L for all others), and DOC (0.5 mg/L).

For OWC analysis, water samples were adjusted to pH 3, spiked with surrogate standards ($^{13}C_3$-caffeine and $^{13}C_2$-17β-estradiol), and then extracted by solid-phase extraction (U.S. EPA method 3535 [31]) using Oasis® hydrophilic-lipophilic-balanced solid phase extraction cartridges (Milford, MA, USA). Samples were not filtered prior to extraction. Since the sample preparation procedure called for solid phase extraction, suspended solids or particulate in the sample was retained on the solid phase disk and extracted during the elution step. Blanks and spiked laboratory controls were prepared using reverse-osmosis water. Following extraction, cartridges were eluted with 5 ml 10% methanol in methyl-*t*-butylether, followed with 5 ml methanol in the same glass culture tube. Extracts were evaporated to near dryness under a stream of nitrogen (<30°C) and then brought to 1-ml volume with 1:1 methanol:deionized H_2O after addition of 10 μl internal standard solution (hydrocodone-d6, bisphenol A-d16, diazepam-d5, and 4-nonylphenol-d8). Finally, samples were filtered into autosampler vials for analysis.

Extracts were analyzed for OWC content using a Thermo TSQ Quantum®, high-pressure liquid chromatography, tandem mass spectrometry system (100 × 2.1 mm, 3 μm C-18 column; Thermo Electron North America, San Jose, CA, USA) and single reaction monitoring mode, with only one parent ion (generally the molecular ion plus hydrogen $[M+H]^+$ ion in positive mode and the $[M-H]^-$ ion in negative mode) allowed through the first quadrupole. The target ion was then fragmented by argon in the collision cell and selected quantitation fragment ions allowed in the third quadrupole. This screening provided greater specificity and sensitivity for the complex pond-water matrices by reducing interferences from organic matter and other components.

The instrument was calibrated using a minimum of five calibration concentrations. Three ionization modes were used to quantify analytes: electrospray ionization mode with negative, positive, or mixed polarity, depending on the analytes (Table 1). These three instrumental analyses allowed a single extract to produce acceptable results for many different compound classes, giving a representative view of pharmaceuticals and personal care products in the pond samples. One transition was used to identify each compound as described previously in Vanderford et al. [29]. While multiple transitions would further corroborate the presence of each compound, a high-

Table 1. Organic wastewater analytes, Chemical Abstracts Service (CAS) reference numbers, detection limits, electrospray ionization polarity, and laboratory control recoveries (mean and standard deviations, SD, $n = 6$, except 4-*n*-nonylphenol for which $n = 2$)

Analyte	CAS no.	Detection limit (ng/L)	Electrospray polarity	Lab controls	
				Mean (%)	SD
Acetaminophen	103-90-2	10	Mixed	99	7.8
Androstenedione	63-05-8	1.0	Positive	117	29
Bisphenol A	80-05-7	36	Negative	103	5.7
Caffeine	58-08-2	16	Positive	111	13
Carbamazepine	298-46-4	0.5	Positive	121	9.2
Diazepam	439-14-5	0.5	Positive	84	5.7
Diclofenac	15307-86-5	2.0	Mixed	93	7.8
Diethylstilbestrol	56-53-1	0.5	Negative	84	8.5
Diethyltoluamide	134-62-3	25	Positive	99	28
17α-Estradiol	57-91-0	0.5	Negative	104	4.9
17β-Estradiol	50-28-2	2.0	Negative	101	7.1
Estriol	50-27-1	2.0	Negative	101	7.1
Estrone	53-16-7	1.0	Negative	104	6.4
17α-Ethynylestradiol	57-63-6	2.0	Negative	102	17
Fluoxetine	54910-89-3	8.0	Mixed	196	21
Gemfibrozil	25812-30-0	0.5	Mixed	91	1.4
Hydrocodone	125-29-1	2.0	Positive	77	4.2
Ibuprofen	15687-27-1	10	Mixed	96	1.4
Iopromide	73334-07-3	20	Mixed	74	5.7
Meprobamate	57-53-4	5.0	Positive	107	18
Naproxen	22204-53-1	2.0	Negative	154	7.1
4-*n*-Nonylphenol	25154-52-3	1.0	Negative	68	4.2
Oxybenzone	131-57-7	940	Positive	117	17
Pentoxifylline	6493-05-6	1.0	Positive	111	8.5
Phenytoin	57-41-0	1.0	Mixed	97	2.8
Progesterone	57-83-0	0.5	Positive	103	21
Sulfamethoxazole	723-46-6	0.5	Positive	87	19
Testosterone	58-22-0	2.0	Positive	113	23
Triclosan	3380-34-5	16	Mixed	103	3.5
Trimethoprim	738-70-5	1.0	Positive	86	4.2

performance liquid chromatography separation with matching retention times of a compound with the correct molecular ion that separates into the correct transition ion at the same collision energy provides sufficient proof of analyte identity. Additionally, recently published U.S. EPA method 1694 also specifies only one transition for identification [32].

Samples for DOC analysis were filtered upon receipt at the laboratory and then preserved with sulfuric acid (pH < 2). Dissolved organic carbon was analyzed using U.S. EPA method 415.1 [33] (persulfate oxidation with infrared detection). Boron was determined using U.S. EPA method 6010B (inductively coupled plasma–optical emission spectroscopy) [34]. Only the August results are reported due to higher detection limits for the February samples (50 μg/L). Nitrate-N was determined both at the laboratory by ion chromatography (U.S. EPA method 300.0 [35]) and in the field using CHEMets® (Chemetrics, Calverton, VA, USA) test kits. Reagents used for CHEMets nitrate analysis in May and August contained a precipitate and thus we are reporting only ion chromatography results for these samples (Table 2). The ion chromatography method reporting limit for nitrate was lowered for August samples to improve detection (0.1 from 0.2 mg/L).

Method quality data

Quality assurance samples were analyzed at least once for each 10 samples and included laboratory and field blanks and laboratory controls (standard-spiked reagent water). Several analytes were detected in laboratory and/or field blanks, including caffeine (two of three field blanks, mean 8.2 ng/L); DEET (two of three field blanks, mean 7.9 ng/L); oxybenzone (three of three field blanks, mean 160 ng/L); fluoxetine (two of three laboratory blanks, mean 2.9 ng/L); and iopromide (once each in laboratory and field blanks, mean 4.5 ng/L). We adjusted reporting limits to account for blank detections as follows: for an analyte detected in more than one blank, the revised reporting limit was determined by adding three times the standard deviation (SD) to the mean of blank values (mean ± 3 SD), or for a single detection, the revised reporting limit was equal to three times the concentration of the blank detection.

On occasion, recoveries of analytes in laboratory controls were out of the acceptable range (i.e., not within 50–150%). Therefore, results for those analytes were not reported for that set of samples. These included acetaminophen (May and August), fluoxetine (February and May), iopromide (May), naproxen (February and August), and testosterone (August).

In the present study, higher DOC concentrations of samples collected in warmer months may have reduced the analytical recovery of surrogate standards, and thus the recovery of compounds they were intended to track. The interference of DOC on extraction efficiency of pesticides from river water has been previously demonstrated [36]. Dissolved organic carbon may also affect ionization processes during instrumental analysis [37]. Recoveries of surrogates were higher overall for LRD samples ($^{13}C_2$-caffeine mean 82%, SD 44%; $^{13}C_2$-17β-estradiol mean 98%, SD 9.4%) than HRD samples ($^{13}C_2$-caffeine mean 47%, SD 34%; $^{13}C_2$-17β-estradiol mean 77%, SD 30%), in which DOC concentrations were on average five times higher. The lowest surrogate recoveries ($^{13}C_2$-caffeine mean 22%, SD 8.9%; $^{13}C_2$-17β-estradiol mean 58%, SD 26%) were measured for August HRD pond samples, which had the highest DOC concentrations.

Table 2. Concentrations of detected hormones, pharmaceuticals, and other contaminants in Cape Cod (Massachusetts, USA) ponds[a]

Category	Compound	Date	Reporting limit	LRD ponds Frequency	LRD ponds Range detected	HRD ponds Frequency	HRD ponds Range detected	Total frequency
Nutrients (mg/L)	DOC	All	0.5	7/7	0.7–1.8	8/8	3.8–8.9	15/15
	NO_3-N[b]	February	0.1	0/2	<0.1	3/3	0.4–0.4	3/5
		May	0.2	1/2	1.0	1/1	0.4	2/3
		August	0.1	0/3	<0.1	0/3	<0.1	0/6
Boron (μg/L)	Boron	August samples only	10	3/3	11–13	4/4	12–250	7/7
Organic wastewater compounds[c] (ng/L)								
Hormones	Androstenedione		1.0	0/7	<1.0	5/9	1.4–3.3	5/16
	17β-Estradiol		2.0	1/7	2.2	0/9	<2.0	1/16
	Estrone		1.0	0/7	<1.0	5/9	1.2–3.0	5/16
	Progesterone		0.5	1/7	0.73	5/9	0.72–6.5	6/16
Pharmaceuticals	Carbamazepine		0.5	0/7	<0.5	3/9	0.63–2.4	3/16
	Ibuprofen		10	1/7	19	0/9	<10	1/16
	Meprobamate		5.0	0/7	<5.0	1/9	7.0	1/16
	Pentoxifylline		1.0	0/7	<1.0	5/9	1.2–2.8	5/16
	Sulfamethoxazole		0.5	1/7	1.5	4/9	0.63–2.2	5/16
	Trimethoprim		1.0	1/7	1.9	3/9	1.4–11	4/16

[a] DOC= dissolved organic carbon; LRD = lower residential density; HRD = higher residential density.
[b] NO_3-N (nitrate-nitrogen) values were determined using CHEMets® (February, Chemetrics, Calverton, VA, USA) and ion chromatography (May, 0.2 mg/L reporting limit; August, 0.1 mg/L reporting limit).
[c] Means were not calculated for organic wastewater contaminants due to the low number of detections.

Dissolved organic carbon from different sources appeared to have a differential effect on analyte recovery. Specifically, even though residential density (e.g., septic discharge) appeared to have a greater impact than season on DOC concentrations (see *Discussion* section below), the DOC associated with summer season (e.g., algal sources) appeared to have a stronger effect on extraction efficiency. For example, surrogate recoveries from February HRD pond water samples, which were high in residential DOC but low in seasonal DOC, were 83% (SD 22%, $^{13}C_2$-caffeine) and 105% (SD 5.9%, $^{13}C_2$-17β-estradiol), values similar to those of LRD pond samples overall but much higher than recovery of surrogates from HRD pond samples in August, when seasonal DOC concentrations were at their peak. This effect was greatest in samples from Lewis Pond, with surrogate recoveries at 17% (SD 13%, $^{13}C_2$-caffeine) and 30% (SD 3.0%, $^{13}C_2$-17β-estradiol). Matrix interference was still pronounced in samples collected in summer from the other two HRD ponds (Oyster and Schoolhouse) for the surrogate $^{13}C_2$-caffeine (26%, SD 4%) but less so for the surrogate $^{13}C_2$-17β-estradiol (76%, SD 4.0%).

RESULTS

In the present study of groundwater-fed ponds vulnerable to contamination by septic leachate, we documented clear differences between profiles of OWCs in samples collected from ponds in areas of higher and lower residential density on Cape Cod. Anticipated seasonal differences due to the threefold increase in population on Cape Cod during summer months were less clear. Because of the small number of samples collected, observed trends by season should be considered speculative. In contrast, the effect of residential density was more clearly demonstrated by data collected. As context for our results on OWCs, we first discuss findings related to nutrients (nitrate and DOC) and boron, constituents known to be associated with wastewater.

Nutrients, boron, and measures of pond heterogeneity

Dissolved organic carbon concentrations in the ponds ranged from 0.7 to 8.9 mg/L (Table 2) and varied with residential density and season (Fig. 2). Of the two factors, residential density was more clearly associated with DOC concentrations, which were approximately five times higher ($p < 0.001$) in HRD ponds (mean 5.5 mg/L; SD 1.9 mg/L) than in LRD ponds (mean 1.0 mg/L; SD 0.4 mg/L), suggesting septic contamination of HRD ponds as the source. Seasonal factors also appeared to influence DOC concentrations in the ponds, though less strongly than residential density, with higher concentrations in samples collected during warmer months (Fig. 2). For the three ponds sampled in both February and August—Joshua, Lewis, and Oyster ponds—DOC concentrations were 41, 65, and 14% higher, respectively, in August than in February. This difference was most likely due to two factors: increased algal production in warmer months and higher septic flows from the large seasonal increase in population.

Trends in NO_3-N concentrations, although generally related to residential density, were dependent on season (Table 2). In both February and May LRD pond samples, NO_3-N concentrations were below the reporting limits, except that in the May sampling round, the duplicate of the single nondetect from an LRD pond (Flax Pond, Dennis) showed an unusually high NO_3-N concentration of 1.8 mg/L (the value of 1.0 that appears in Table 2 is the average of this duplicate and the original nondetect). In each HRD pond sample collected in February and May, the NO_3-N concentration was 0.4 mg/L. In contrast, in all August samples from both LRD and HRD ponds, NO_3-N concentrations were below the reporting limit (<0.1 mg/L). These results may reflect enhanced denitrification favored by conditions typical of ponds in August, including higher DOC concentrations, higher temperatures, and lower dissolved oxygen [38,39].

Boron was used as an indicator of wastewater influence because of its presence in detergents. August boron concentrations were fairly uniform in the three LRD ponds (mean 11.7 μg/L; SD 1.1 μg/L) but varied substantially in HRD ponds, from 12 μg/L in Schoolhouse Pond to 250 μg/L in Oyster Pond. High boron concentrations in seawater [40] probably account for the concentrations in the brackish Oyster

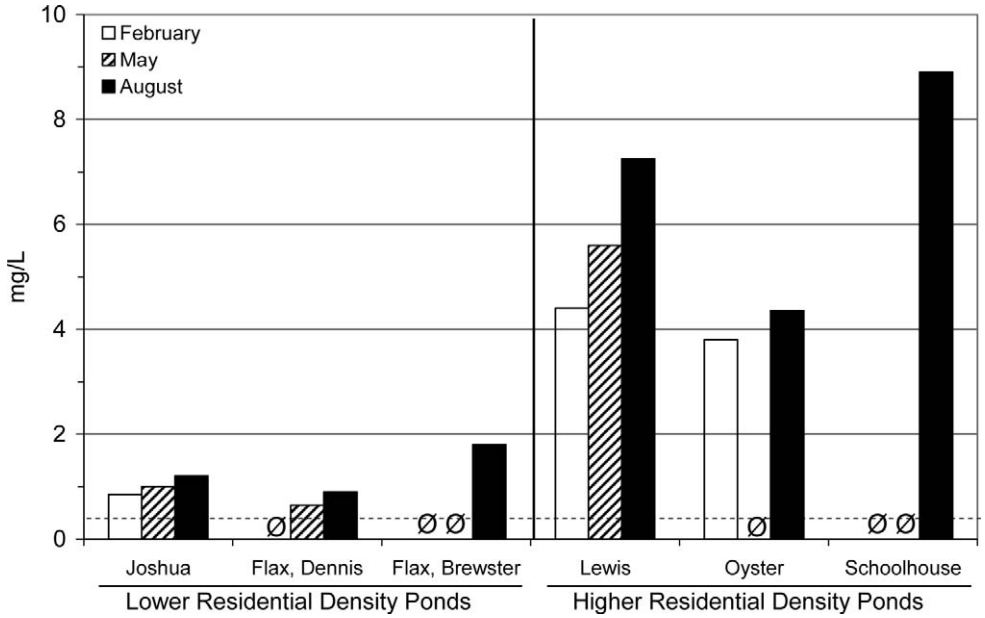


Fig. 2. Dissolved organic carbon concentrations in pond water samples collected in lower and higher residential density ponds in February (stippled bar), May (diagonal striped bar), and August (black bar), 2006. The dashed line shows the reporting limit. Not all ponds were sampled in all seasons; the symbol (∅) indicates that no sample was collected.

Pond. Because the other five ponds were located 1 to 7 km away from the ocean and the general direction of groundwater flow was toward the ocean, we considered the possibility of sea-spray and brackish groundwater–derived boron to these ponds to be unlikely. In future studies, isotopic analyses may allow differentiation of seawater and wastewater contributions [41]. In Lewis Pond, boron was detected at 22 μg/L in August samples, twice the mean concentrations measured in LRD ponds and 10% of those measured by Swartz et al. [15] in septage, suggesting wastewater contribution.

Physicochemical heterogeneity in Lewis Pond was determined in August 2007 by measuring temperature, conductivity, dissolved oxygen, and pH at six horizontally spaced locations (east, west, south, center, and along a transect between the southern shore and the center of the pond), as well as two additional depths at the central location (data not shown). These measures do not provide direct information on the heterogeneity in hormone and pharmaceutical concentrations in this or other study ponds or during other years or seasons, such as during thermocline formation in summer, or take into account factors such as photolysis and microbial degradation, but they do give some indication of the extent of mixing in a pond immediately surrounded by approximately 20 residences serviced by on-site septic systems (additional houses are located nearby).

Of the four parameters measured at varying depths and across horizontal transects in Lewis Pond, pH varied the least (mean 7.3, SD 0.3) and dissolved oxygen varied the most (mean 9.7 mg/L, SD 1.3 mg/L). Results for conductivity (mean 108 μS/cm, SD 6 μS/cm) and temperature (mean 26.6°C, SD 2.7°C) had intermediate variability. For these parameters, mean values of samples collected across horizontal transects were within 0 to 4% of means for samples collected at different depths, indicating that the pond was well mixed horizontally and vertically at the time of data collection. Conductivity values, likely the best indicator for wastewater contamination in the pond, fell within a narrow range between 98 and 113 μS/cm for samples collected at different depths and 98 to 111 μS/cm for samples collected across the horizontal transects. As discussed further below, differences between OWC concentrations in samples collected on the same day from different locations were within 1 ng/L, also indicating that ponds were well mixed and samples collected for the present study representative.

Organic wastewater compounds

Prevalence of OWCs. As detailed above, we analyzed 16 water samples from six Cape Cod ponds for 29 OWCs: seven endogenous hormones, 18 pharmaceuticals, and four other compounds, including bisphenol A and triclosan. Ten of these compounds were measured at least once above reporting limits, at concentrations up to 19 ng/L (summary results in Table 2). Field blanks contained low levels of several compounds, including caffeine, DEET, and oxybenzone, in spite of efforts to avoid use of potentially contaminating sources such as caffeinated beverages, insect sprays, and sunscreen during sampling. It is possible that the boat used for sampling was not sufficiently cleaned prior to use.

In all, three hormones (androstenedione, estrone, and progesterone) and four pharmaceuticals (carbamazepine, pentoxifylline, sulfamethoxazole, and trimethoprim) were detected three to six times; in addition, 17β-estradiol, ibuprofen, and meprobamate were each detected once. There was generally good agreement among samples collected from different locations in the same pond (>100 m apart), with 1 ng/L or less difference between sample results, consistent with the limited variation observed for other parameters in Lewis pond in the summer of 2007 and suggesting that the ponds were fairly well mixed laterally.

Contaminants and residential density. Of the 29 OWCs analyzed, the total number detected in HRD pond samples (mean 3.5 compounds per sample, SD 0.5) was significantly higher ($p < 0.001$) than the number detected in LRD ponds (mean 0.7 compounds per sample, SD 1.2). These findings are consistent with the results of a recent study by Lee et al. [42], who found more OWCs in rivers influenced by wastewater

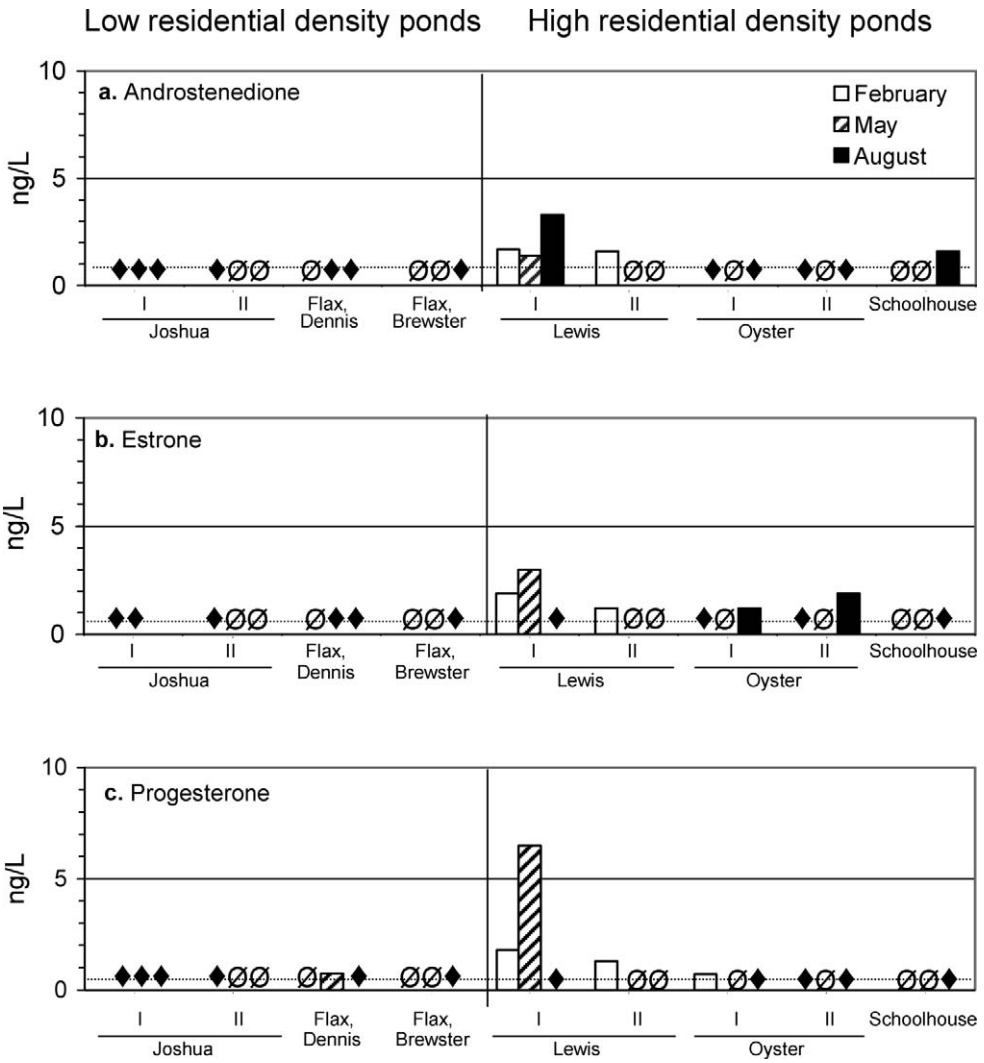


Fig. 3. Concentrations of selected steroidal hormones in pond water samples collected in lower and higher residential density ponds in February (stippled bar), May (diagonal striped bar), and August (black bar), 2006. Dashed lines show reporting limits. Ponds are identified along the *x* axis. Samples labeled I and II were collected on the same date from separate locations within ponds. Not all ponds were sampled in all seasons; the symbol (♦) represents a value below the reporting limit and the symbol (∅) indicates that no sample was collected.

(mean 8.9 OWCs per sample, of 97 compounds analyzed) than in rivers not receiving wastewater (mean 3.6 per sample) or in groundwater (mean 3.0 per sample).

Of the compounds examined in the present study, the hormones androstenedione, estrone, and progesterone were detected more frequently in HRD than LRD pond samples (Table 2, Fig. 3a–c). All HRD pond samples contained at least one steroidal hormone, and three of the four samples collected from Lewis Pond contained all three hormones analyzed (Fig. 3a–c). In contrast, detections of hormones were rare in LRD pond samples, with 17β-estradiol and progesterone each detected once in May, in different ponds (Fig. 3c; 17β-estradiol not shown).

In a study on migration of OWCs in a wastewater plume, Swartz et al. [15] noted that estrone is more persistent in groundwater than 17β-estradiol. These researchers observed a substantial decrease in 17β-estradiol, but not estrone, in groundwater over a 5.5-m traverse downgradient of a septic leach pit. Consistent with those findings, we detected estrone, but not 17β-estradiol, in groundwater-fed HRD ponds. The source of the single detection of 17β-estradiol in Joshua Pond is unclear but the pond is used extensively for recreation by both humans and dogs.

Hormones were also detected at higher concentrations in HRD ponds than in LRD ponds. Androstenedione, estrone, and progesterone were measured at concentrations up to 3.3, 3.0, and 6.5 ng/L, respectively in HRD ponds. Two of these hormones, androstenedione and estrone, were present at significantly higher concentrations in HRD ponds than in LRD ponds ($p < 0.05$). Because DOC concentrations associated with seasonal sources (such as algal excretions) appeared to interfere with extraction efficiency, particularly in Lewis Pond, we corrected steroidal hormone results for surrogate recovery ($^{13}C_2$-17β-estradiol) in these samples. With this correction, concentrations of androstenedione, estrone, and progesterone in May samples collected in Lewis Pond increased from 1.4, 3.0, and 6.5 ng/L to 2.2, 4.6, and 10.0 ng/L, respectively. Similarly, the concentration of androstenedione in the August Lewis Pond sample increased from 3.3 to 11 ng/L after correction for surrogate recovery.

Prevalence and concentrations of pharmaceuticals show similar correspondence with residential density. We detected

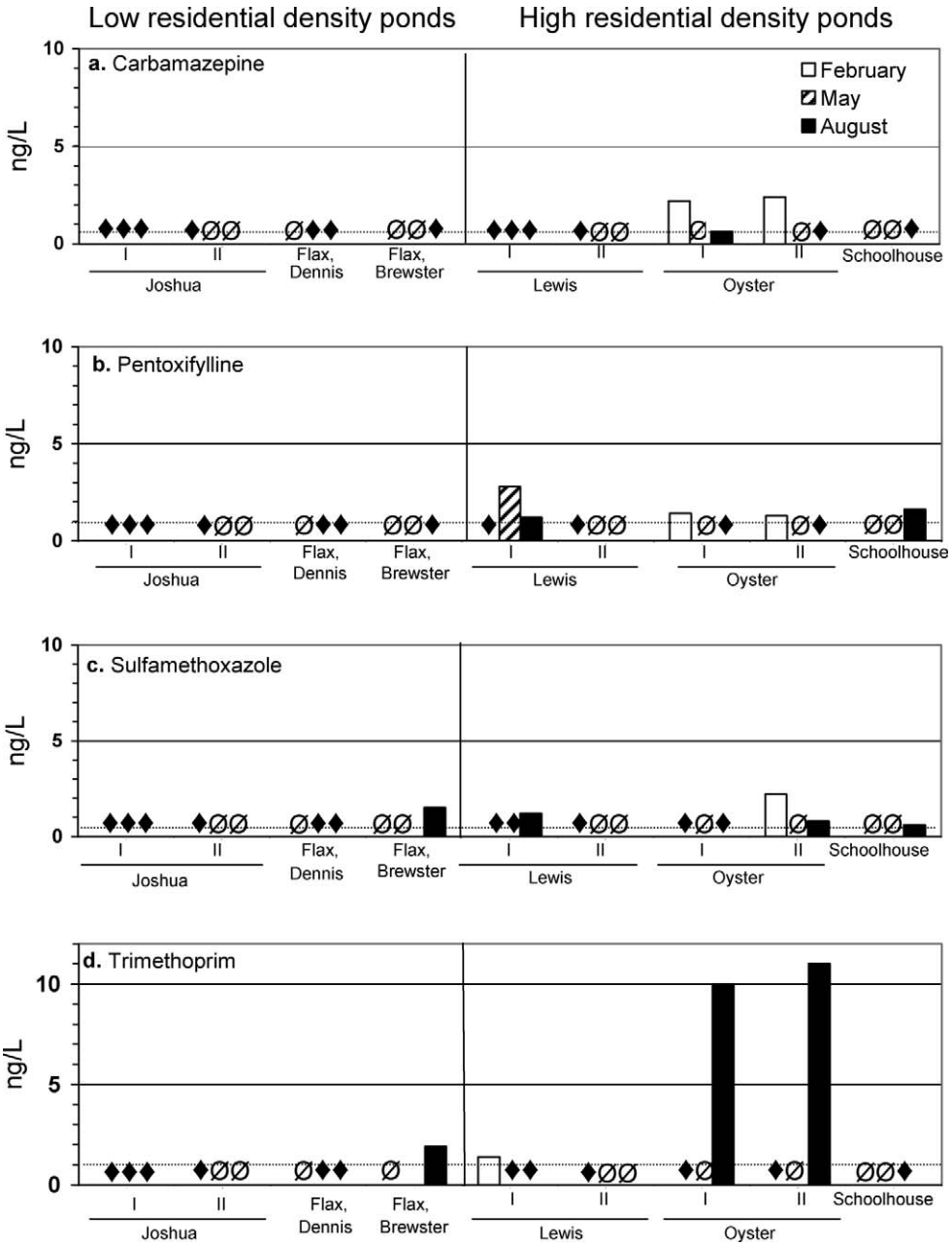


Fig. 4. Concentrations of selected pharmaceuticals in pond water samples collected in lower and higher residential density ponds in February (stippled bar), May (diagonal striped bar), and August (black bar), 2006. Dashed lines show reporting limits. Ponds are identified along the *x* axis. Samples labeled I and II were collected on the same date from separate locations within ponds. Not all ponds were sampled in all seasons; the symbol (♦) represents a value below the reporting limit and the symbol (∅) indicates that no sample was collected.

pharmaceuticals more frequently and at higher concentrations in HRD than in LRD pond samples (Fig. 4a–d; Table 2); the difference in concentrations was statistically significant only for pentoxifylline ($p < 0.05$). The pharmaceuticals detected most often were carbamazepine, pentoxifylline, sulfamethoxazole, and trimethoprim, at concentrations up to 2.4, 2.8, 2.2, and 11 ng/L, respectively. Meprobamate and ibuprofen were detected only once each in different ponds: meprobamate at 7.0 ng/L in an HRD pond (Schoolhouse Pond; August) and ibuprofen at 19 ng/L in an LRD pond (Flax Pond, Brewster; August). The latter was the only LRD pond sample with detectable concentrations of pharmaceuticals (ibuprofen, sulfamethoxazole, and trimethoprim); the ibuprofen concentration in this sample (19 ng/L) was the highest of all detected OWCs in the present study (Table 2). This may reflect the intensive use of this pond for recreational activities, such as swimming and camping, during the summer months.

Caffeine is often utilized as a tracer of wastewater [13,43] and has been detected frequently in surface waters influenced by wastewater [7,8], but was not detected above the reporting limit (16 ng/L) in the present study. Several factors may contribute to this: detection in blanks elevating the reporting limit; reduced surrogate recovery in HRD samples (mean 47%); and likely biodegradation of caffeine during transit. For example, Swartz et al. [15] noted that caffeine was removed more efficiently between a septic tank and downgradient monitoring wells than compounds such as estradiol, estrone, and fluorescent whitening agents.

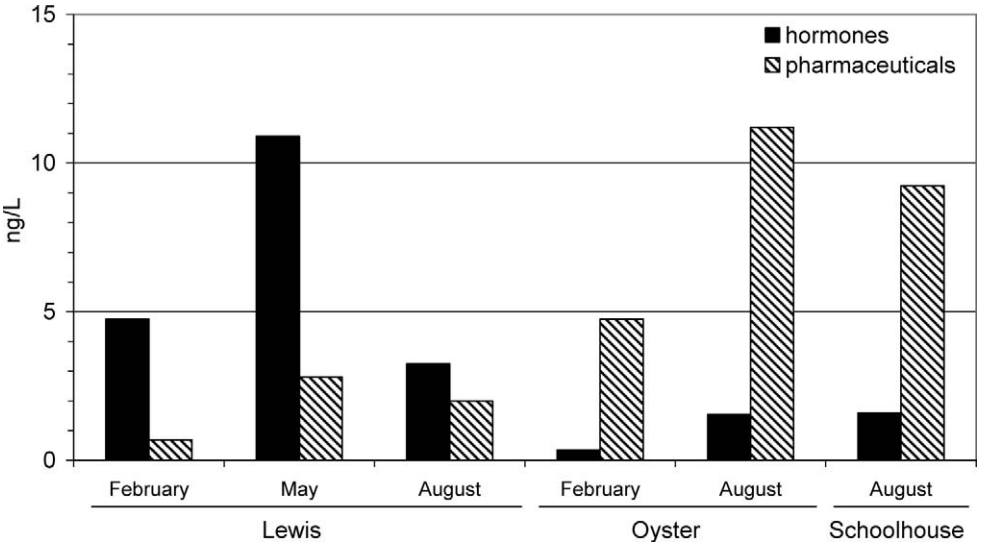


Fig. 5. Sums of concentrations of steroidal hormones and pharmaceuticals in pond samples on Cape Cod (Massachusetts, USA) collected during all seasons in the higher residential density ponds.

OWCs and season. We expected that seasonal shifts in concentrations of OWCs might occur in study ponds as larger summer populations on Cape Cod would result in both increased wastewater flow to septic systems and greater in-pond contributions from recreation. Although, as discussed previously, DOC concentrations increased and NO_3-N concentrations decreased in warmer months, no clear seasonal trend was evident in OWC concentrations for the two HRD ponds (Lewis and Oyster ponds) sampled in both February and August. Steroidal hormones in Lewis Pond samples were generally higher in warmer months; similarly, estrone was detected only in August in Oyster pond samples (Fig. 3a–c). In contrast, progesterone—a steroid measured in three of four samples collected in February from HRD ponds—was not detected in samples collected from any of the six ponds in August (Fig. 3c). Moreover, there was no overall seasonal pattern for pharmaceuticals (Fig. 4a–d). Possible reasons for this lack of seasonal trend, such as groundwater migration rates delaying arrival of effluent to ponds beyond our August sample time and reduced rates of transformation in cooler months [44], will be investigated in future studies.

Differences in OWC profiles across ponds. As described above, both hormones and pharmaceuticals were detected in samples from all three HRD ponds. However, the relative frequency of pharmaceutical and hormone detections varied by pond, and each pond's profile was consistent across seasons (Fig. 5). Specifically, hormones dominate in samples from Lewis Pond (62–87% of ΣOWC concentration), whereas pharmaceuticals dominate in samples from Oyster Pond (88–93%). Samples collected from Schoolhouse Pond were also dominated by pharmaceuticals (85%), but this HRD pond was only sampled once (August) and thus requires further study to determine whether this pattern holds across seasons.

There are at least two possible explanations for the differences in OWC profiles across ponds. First, demographics may play a role. For example, if senior citizens use greater quantities of pharmaceuticals, a senior condominium complex located on the upgradient shore of Oyster Pond may affect its OWC profile. However, it is difficult to determine which residences are contributing, because patterns of groundwater flow are complex. For example, mounding of effluent below septic systems may distort groundwater flow, allowing lateral contributions to the pond from residences located near the shore but not upgradient of the pond. Indeed, an initial survey to track the flux of wastewater into Oyster Pond using fluorescence as an indicator ([45]; www.opet.org, accessed June 25, 2008) documented just such a pattern.

Second, there may be nonwastewater sources of the steroidal hormones β-estradiol, estrone, and progesterone, due to recreational use of the ponds by people and dogs [46]. Additionally the compound androsterone, a reduced form of androstenedione, is a component of deer deterrents (www.freepatentsonline.com/4451460.html, accessed March 8, 2007) that may be used by gardeners in the area to protect vegetation from grazing deer. Therefore, surface runoff may contribute this chemical to the ponds.

DISCUSSION

Comparison to other studies

In the literature to date on OWCs in water resources, ponds on Cape Cod are unique because surface water bodies are fed primarily by aquifers receiving wastewater from on-site septic systems rather than from treatment plant effluent. However, recent reports on OWC contamination of groundwater in the vicinity of septic systems provide some context for our results.

Swartz et al. [15] detected 17β-estradiol and estrone at concentrations ranging from 0.3 to 3.0 ng/L and 20 to 23 ng/L, respectively, in groundwater water collected 5.5 m downgradient from a septic leach pit on Cape Cod. The one detection of 17β-estradiol in the present study was within the range measured by these authors, but estrone concentrations in that study were an order of magnitude higher than concentrations detected in the present study (Table 2).

In a study of well water collected from an aquifer underlying a Montana region undergoing rapid residential development serviced by onsite wastewater disposal, Miller and Meek [19] (www.deq.state.mt.us/wqinfo/pws/docs/Helena%20valley%20pharms_new.pdf, accessed June 25, 2008) detected 17β-estradiol, estrone, and progesterone infrequently (2–4% of samples) at concentrations ranging from 0.6 to 2.9 ng/L, values similar to those detected in the

present study. In their study, pharmaceuticals were detected more frequently and at higher concentrations than hormones, with carbamazepine detected in 31% of samples (median <0.5 ng/L, maximum 420 ng/L) and sulfamethoxazole detected in 78% (median 3.1 ng/L; maximum 490 ng/L) [19]. Godfrey et al. [17] also studied groundwater contamination by septic effluent in Montana and detected pharmaceuticals such as carbamazepine and sulfamethoxazole at concentrations ranging from tens to hundreds of nanograms per liter and at distances up to 15 m downgradient of a high school septic drain field. Finally, in a region of eastern Nebraska where 61% of households use on-site septic systems, Verstraeten et al. [20] measured acetaminophen, sulfamethoxazole, and trimethoprim in 8 to 26% of domestic well water samples. Hormones were not measured in that study.

Two U.S. Geological Survey studies of surface water contamination provide additional context, although in these studies the source of most wastewater was treatment plant effluent [7,8]. The first study reported reproductive hormones in 3 to 21% of samples at median concentrations up to approximately 100 ng/L [7]. Both studies reported a large number of pharmaceuticals detected: 23 of 41 analyzed [7] and 17 of a similar but not identical set of 41 compounds [8]. Among the pharmaceuticals reported were sulfamethoxazole at median concentrations of 66 to 150 ng/L and trimethoprim at 11 to 150 ng/L [7,8]. Overall, the median concentrations of hormones and pharmaceuticals measured in those two surveys [7,8] were higher than concentrations measured in pond samples of the present study.

Biological implications. Detection of biologically active hormones and pharmaceuticals in Cape Cod ponds raises concern regarding the potential impact on aquatic species living in these ecosystems. As a case in point, the incidence of intersex fish caught wild in rivers in the United Kingdom has been directly related to the proportion of wastewater in those rivers [2]. Laboratory studies have identified a number of OWCs that may be responsible for these reproductive effects. For example, Metcalfe et al. [3] detected slightly elevated incidence of intersex condition of Japanese medaka (*Oryzias latipes*) when these fish were exposed to 10 ng/L estrone or 17β-estradiol. Tabata et al. [4] detected female-specific proteins in all of five male medaka after five weeks of exposure to 5 ng/L 17β-estradiol.

In the present study, the estrogenic hormones estrone and progesterone were measured in ponds on Cape Cod at concentrations up to 3 and 6.5 ng/L, respectively (4.6 and 10 ng/L, respectively, when corrected for surrogate recovery); these concentrations approach those observed to induce physiological responses in fish in the studies discussed above.

Fewer studies have been conducted on the ecological implications of pharmaceuticals as context for our results. In one study, an exposure of 1 μg/L ibuprofen resulted in reduced growth of the aquatic plant *Lemna minor* [47]. However, that concentration is two orders of magnitude higher than the ibuprofen concentration we measured.

Although measures of ecotoxicological responses to individual compounds are informative, OWCs most often occur as mixtures, in Cape ponds and elsewhere. As discussed above, we measured an average of more than three compounds co-occurring in HRD pond samples. Mixtures of compounds may exert synergistic, additive, or antagonistic effects. For example, Brian et al. [48] compared induction of the female reproductive protein vitellogenin in male fathead minnows exposed to the estrogens 17β-estradiol and ethynylestradiol, and the estrogenic compounds nonylphenol, octylphenol, and bisphenol A, first as individual compounds and then as a mixture. While the individual compounds had no effect at concentrations one-fifth their median effective concentration, a mixture of the five compounds, each at one-fifth their median effective concentration, induced vitellogenin production in the fish, indicating an additive effect of the endogenous and xenobiotic estrogens. Similarly, Thorpe et al. [49] measured an additive effect of 17β-estradiol and nonylphenol on plasma vitellogenin concentrations in juvenile rainbow trout (*Oncorhynchus mykiss*). Measures of the aquatic toxicity of 10 pharmaceuticals to the crustacean *Daphnia magna* and the plant *L. minor* indicated additive effects of pharmaceuticals with similar modes of action and independent effects of pharmaceuticals with dissimilar modes of action [50].

CONCLUSION

The present study demonstrates that surface water ecosystems fed by groundwater, impacted in turn by effluent from residential on-site wastewater treatment systems, are susceptible to contamination by estrogenic and other biologically active OWCs, much as rivers are affected by point-source discharges of effluent from wastewater treatment. The primary difference between the two sets of ponds in the present study, and thus the primary source of these contaminants, was the presence of residential septic systems in their watershed. Such considerations should inform land-use planning and policy, particularly in areas where development outstrips the construction of sewage infrastructure. Furthermore, surface water bodies such as the ponds studied here can act as regional indicators of groundwater contamination. In areas such as Cape Cod, where aquifers are the sole or primary source for drinking water, this contamination has implications for human health.

Acknowledgement—This work was primarily supported by grants from Massachusetts Environmental Trust, Babylon Breast Cancer Coalition, the Agua Fund of the Tides Foundation, and the Susan S. Bailis Breast Cancer Research Fund. We also appreciate assistance with sampling by Mark Benotti, Laura Gerber, Farley Lewis, Megan McAuliffe, and coordination of Cape sampling efforts by Cheryl Osimo.

REFERENCES

1. Purdom CE, Hardiman PA, Bye VJ, Eno NC, Tyler CR, Sumpter JP. 1994. Estrogenic effects of effluents from sewage treatment works. *Chem Ecol* 8:275–285.
2. Jobling S, Nolan M, Tyler CR, Brighty G, Sumpter JP. 1998. Widespread sexual disruption in wild fish. *Environ Sci Technol* 32:2498–2506.
3. Metcalfe CD, Metcalfe TL, Kiparissis Y, Koenig BG, Khan C, Hughes RJ, Croley TR, March RE, Potter T. 2001. Estrogenic potency of chemicals detected in sewage treatment plant effluents as determined by in vivo assays with Japanese medaka (*Oryzias latipes*). *Environ Toxicol Chem* 20:297–308.
4. Tabata A, Kashiwada S, Ohnishi Y, Ishikawa H, Miyamoto N, Itoh M, Magara Y. 2001. Estrogenic influences of estradiol-17β, *p*-nonylphenol and bisphenol-A on Japanese medaka (*Oryzias latipes*) at detected environmental concentrations. *Water Sci Technol* 43:109–116.
5. Brody JG, Rudel RA. 2003. Environmental pollutants and breast cancer. *Environ Health Perspect* 111:1007–1019.
6. Buser H-R, Müller MD, Theobald N. 1998. Occurrence of the pharmaceutical drug clofibric acid and the herbicide mecoprop in various Swiss lakes and in the North Sea. *Environ Sci Technol* 32:188–192.

7. Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, Barber LB, Buxton HT. 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999–2000: A national reconnaissance. *Environ Sci Technol* 36:1202–1211.
8. Glassmeyer ST, Furlong ET, Kolpin DW, Cahill JD, Zaugg SD, Werner SL, Meyer MT, Kryak DD. 2005. Transport of chemical and microbial compounds from known wastewater discharges: Potential for use as indicators of human fecal contamination. *Environ Sci Technol* 39:5157–5169.
9. Buerge IJ, Poiger T, Muller MD, Buser H-R. 2003. Caffeine, an anthropogenic marker for wastewater contamination of surface waters. *Environ Sci Technol* 37:691–700.
10. Heberer T. 2002. Tracking persistent pharmaceutical residues from municipal sewage to drinking water. *J Hydrol* 266:175–189.
11. Tixier C, Singer HP, Oeller S, Muller SR. 2003. Occurrence and fate of carbamazepine, clofibric acid, diclofenac, ibuprofen, ketoprofen, and naproxen in surface waters. *Environ Sci Technol* 37:1061–1068.
12. Rudel RA, Melly SJ, Geno PW, Sun G, Brody JG. 1998. Identification of alkylphenols and other estrogenic phenolic compounds in wastewater, septage, and groundwater on Cape Cod, Massachusetts. *Environ Sci Technol* 32:861–869.
13. Seiler RL, Zaugg SD, Thomas JM, Howcroft DL. 1999. Caffeine and pharmaceuticals as indicators of waste water contamination in wells. *Ground Water* 37:405–410.
14. Hinkle SR, Weick RJ, Johnson JM, Cahill JD, Smith SG, Rich BJ. 2005. Organic wastewater compounds, pharmaceuticals, and coliphage in ground water receiving discharge from onsite wastewater treatment systems near La Pine, Oregon: Occurrence and implications for transport. Scientific Investigation Report 2005-5055. Report. U.S. Geological Survey, Reston, VA.
15. Swartz CH, Reddy S, Benotti MJ, Yin H, Barber LB, Brownawell BJ, Rudel RA. 2006. Steroid estrogens, nonylphenol ethoxylate metabolites, and other wastewater contaminants in groundwater affected by a residential septic system on Cape Cod, MA. *Environ Sci Technol* 40:861–869.
16. Barber LB, Thurman EM, Schroeder MP, LeBlanc D. 1988. Long-term fate of organic micropollutants in sewage-contaminated groundwater. *Environ Sci Technol* 22:205–211.
17. Godfrey E, Woessner WW, Benotti MJ. 2007. Pharmaceuticals in on-site sewage effluent and ground water, western Montana. *Ground Water* 45:263–271.
18. Cole ML, Kroeger KD, McClelland JW, Valiela I. 2006. Effects of watershed land use on nitrogen concentrations and δ^{15} nitrogen in groundwater. *Biogeochemistry* 77:199–215.
19. Miller KJ, Meek J. 2006. Helena Valley ground water: Pharmaceuticals, personal care products, endocrine disruptors (PPCPs) and microbial indicators of fecal contamination. Report. Montana Department of Environmental Quality, Helena, MT.
20. Verstraeten IM, Fetterman GS, Meyer MT, Bullen T, Sebree SK. 2005. Use of tracers and isotopes to evaluate vulnerability of water in domestic wells to septic waste. *Ground Water Monit Remediat* 25:107–117.
21. Conn KE, Barber LB, Brown GK, Siegrist RL. 2006. Occurrence and fate of organic contaminants during onsite wastewater treatment. *Environ Sci Technol* 40:7358–7366.
22. Carrara C, Ptacek CJ, Robertson WD, Blowes DW, Moncur MC, Sverko E, Backus S. 2008. Fate of pharmaceutical and trace organic compounds in three septic system plumes, Ontario, Canada. *Environ Sci Technol* 42:2805–2811.
23. Moore JW, Schindler DE, Scheuerell MD, Smith D, Frodge J. 2003. Lake eutrophication at the urban fringe, Seattle Region, USA. *Ambio* 32:13–18.
24. Westgate EJ, Kroeger KD, Pabich WJ, Valiela I. 2000. Fate of anthropogenic nitrogen in a nearshore Cape Cod aquifer. *Biol Bul* 199:221–223.
25. Peeler KA, Opsahl SP, Chanton JP. 2006. Tracking anthropogenic inputs using caffeine, indicator bacteria, and nutrients in rural freshwater and urban marine systems. *Environ Sci Technol* 40: 7616–7622.
26. Eichner EM, Cambareri TC, Belfit G, McCaffery D, Michaud S, Smith B, Fenn M. 2003. Cape Cod Pond and Lake Atlas. Cape Cod Commission, Barnstable, MA, USA.
27. Garabedian SP, LeBlanc D, Gelhar LW, Celia MA. 1991. Large-scale natural gradient tracer test in sand and gravel, Cape Cod, Massachusetts: 2. Analysis of spatial moments for a nonreactive tracer. *Water Resour Res* 27:911–924.
28. U.S. Environmental Protection Agency. 2001. Ambient water quality criteria recommendations: Information supporting the development of state and tribal nutrient criteria for lakes and reservoirs in nutrient ecoregion XIV. Technical Report. EPA/822/b-01/011. Washington, DC.
29. Vanderford BJ, Pearson RA, Rexing DJ, Snyder SA. 2003. Analysis of endocrine disruptors, pharmaceuticals, and personal care products in water using liquid chromatography/tandem mass spectrometry. *Anal Chem* 75:6274–6285.
30. U.S. Environmental Protection Agency. 1983. Methods for chemical analysis of water and wastes. EPA method 150.2. Technical Report. EPA/600/4-79/020. Cincinnati, OH.
31. U.S. Environmental Protection Agency. 1996. Test methods for evaluating solid waste, EPA method 3535, SW-846, 3rd ed, update III, revision 0. Washington, DC.
32. U.S. Environmental Protection Agency. 2007. Pharmaceuticals and personal care products in water, soil, sediment, and biosolids by HPLC/MS/MS. EPA Method 1694. Technical Report. EPA/821/r-08/002. Washington, DC.
33. U.S. Environmental Protection Agency. 1983. Organic carbon, total. EPA Method 415.1. Cincinnati, OH.
34. U.S. Environmental Protection Agency. 1996. Test methods for evaluating solid waste. EPA method 6010B, SW-846, 3rd ed, update III, revision 2. Washington, DC.
35. U.S. Environmental Protection Agency. 1993. Determination of inorganic anions by ion chromatography. EPA method 300.0, revision 2.1. Washington, DC.
36. Johnson WE, Fendinger NJ, Plimmer JR. 1991. Solid-phase extraction of pesticides from water: Possible interferences from dissolved organic material. *Anal Chem* 63:1510–1513.
37. Steen RJCA, Hogenboom AC, Leonards PEG, Peerboom RAL, Cofino WP, Brinkman UAT. 1999. Ultra-trace-level determination of polar pesticides and their transformation products in surface and estuarine water samples using column liquid chromatography-electrospray tandem mass spectrometry. *J Chrom* 857:157–166.
38. Senn DB, Hemond HF. 2002. Nitrate controls on iron and arsenic in an urban lake. *Science* 296:2372–2376.
39. Appelboom TW, Chescheir GM, Skaggs RW, Gilliam JW, Amatya D. 2006. Temperature coefficient for modeling denitrification in surface water sediments using the mass transfer coefficient. Hydrology and Management of Forested Wetlands, *Proceedings*, International Congress of the Association of Agricultural and Biological Engineers. St. Joseph, MI, USA, April 8-12, pp. 199–207.
40. Gast JA, Thompson TG. 1958. Determination of the alkalinity and borate concentration of sea water. *Anal Chem* 30:1549.
41. Vengosh A, Weinthal E, Kloppmann W. 2004. Natural boron contamination in Mediterranean groundwater. *Geotimes* 49:20–25.
42. Lee KE, Barber LB, Furlong ET, Cahill JD, Kolpin DW, Meyer MT, Zaugg SD. 2004. Presence and distribution of organic wastewater compounds in wastewater, surface, ground, and drinking waters, Minnesota, 2000-02. Scientific Investigations Report 2004–5138. Report. U.S. Geological Survey, Reston, VA.
43. Standley LJ, Kaplan LA, Smith D. 2000. Molecular tracers of organic matter sources to surface water resources. *Environ Sci Technol* 34:3124–3130.
44. Vieno NM, Tuhkanen T, Kronberg L. 2005. Seasonal variation in the occurrence of pharmaceuticals in effluents from a sewage treatment plant and in the recipient water. *Environ Sci Technol* 39:8220–8226.
45. Soltanoff C, Cheney L, Mach M. 2004. Oyster Pond: Concentrations, effects and sources of groundwater nutrients. Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA, USA.
46. Olson PN, Bowen RA, Behrendt MD, Olson JD, Nett TM. 1982. Concentrations of reproductive hormones in canine serum throughout late anestrus, proestrus and estrus. *Biol Reprod* 27: 1196–1206.
47. Pomati F, Netting AG, Calamari D, Neilan BA. 2004. Effects of erythromycin, tetracycline and ibuprofen on the growth of *Synechocystis* sp. and *Lemna minor*. *Aquat Toxicol* 67:387–396.
48. Brian JV, Harris CA, Scholze M, Backhaus T, Booy P, Lamoree M, Pojana G, Jonkers N, Runnalls T, Bonfa A, Marcomini A, Sumpter JP. 2005. Accurate prediction of the response of fresh-

water fish to a mixture of estrogenic chemicals. *Environ Health Perspect* 113:721–728.

49. Thorpe KL, Hutchinson TH, Hetheridge MJ, Scholze M, Sumpter JP, Tyler CR. 2001. Assessing the biological potency of binary mixtures of environmental estrogens using vitellogenin induction in juvenile rainbow trout (*Oncorhynchus mykiss*). *Environ Sci Technol* 35:2476–2481.
50. Cleuvers M. 2003. Aquatic ecotoxicity of pharmaceuticals including assessment of combination effects. *Toxicol Lett* 142:185–194.