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Baseline

Caffeine and agricultural pesticide concentrations in surface water and groundwater on the north shore of Kauai (Hawaii, USA)

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ABSTRACT

Caffeine has been associated with wastewater pollution in temperate and subtropical locations, but environmental caffeine concentrations in tropical locations have not been reported. The objectives of this study were to measure caffeine and agricultural pesticide (carbaryl, metalaxyl, and metribuzin) concentrations in environmental waters on the tropical north shore of Kauai (Hawaii, USA) and assess whether patterns in caffeine concentration were consistent with a wastewater caffeine source. Groundwater, river, stream and coastal ocean samples were collected in August 2006 and February 2007. Caffeine was detected in all August 2006 samples and in 33% of February 2007 samples at concentrations up to 88 ng L^{-1}. Metribuzin was detected in five samples collected in February 2007. Carbaryl and metalaxyl were not detected in any sample. Caffeine was not detected in offshore ocean samples or river samples upstream of human development. A positive correlation between caffeine and enterococci suggested a possible wastewater caffeine source.

Caffeine ($C_8H_{10}N_4O_2$), a xanthine alkaloid compound and central nervous system stimulant, is the most widely used psychoactive drug in the world. About 90% of North American adults consume caffeine daily, mainly in the form of coffee, tea, or caffeinated soft drinks (Lovett, 2005). Approximately 3% of a person's caffeine intake is excreted in the urine (Tang-Liu et al., 1983). Thus, caffeine can enter the wastewater stream either in urine or when caffeine-containing products, such as beverages or pharmaceuticals, are disposed of through household plumbing and sewer systems (Seiler et al., 1999). Studies in the mainland United States (Siegener and Chen, 2002; Peeler et al., 2006), Europe (Buerge et al., 2003; Weigel et al., 2004) and Australia (Chen et al., 2002) have linked caffeine concentrations in ground and surface waters to wastewater contamination and suggested that caffeine could be used as a wastewater tracer. However, to the best of our knowledge, environmental caffeine concentrations in tropical regions have not been reported.

This study aimed to address that gap by measuring environmental caffeine concentrations on the north shore of Kauai (Fig. 1), a tropical, relatively undeveloped area. Maintaining good water quality is essential for the health of the north shore's coral reefs, human swimmers, and the tourism-based economy. In 2007, the most recent year for which complete data were available, over 1.3 million tourists visited Kauai, with 70% spending time at the beach and 37% snorkeling or SCUBA diving (State of Hawaii, 2007).

Intermittently high levels of enterococci, the fecal indicator bacteria used to set water quality standards in the United States and the state of Hawaii, have been detected in beach and river water on the north shore of Kauai since 2000 (Hanalei Watershed Hui, unpublished data), raising concerns about sewage contamination. Except for the Princeville community east of the Hanalei River (Fig. 1), which has a wastewater treatment plant (WWTP), the north shore relies on septic systems and cesspools. Previous work (Knee et al., 2008) suggested that fecal indicator bacteria enter beach water mainly via rivers and streams and not from groundwater discharge or contaminated sand. However, whether waterborne enterococci originate from human sewage or the feces of wild or domestic animals remains unknown. Naturally occurring enterococci bacteria have also been reported in Hawaiian (Hardina and Fujioka, 1991) and Guamanian (Fujioka et al., 1999) soils and streams, suggesting that they may not always be associated with fecal pollution in these, and likely other, tropical locations.

Novel wastewater tracers, such as caffeine, may be useful for identifying fecal pollution in tropical settings where traditional indicator bacteria grow naturally. Caffeine may also be a pollutant

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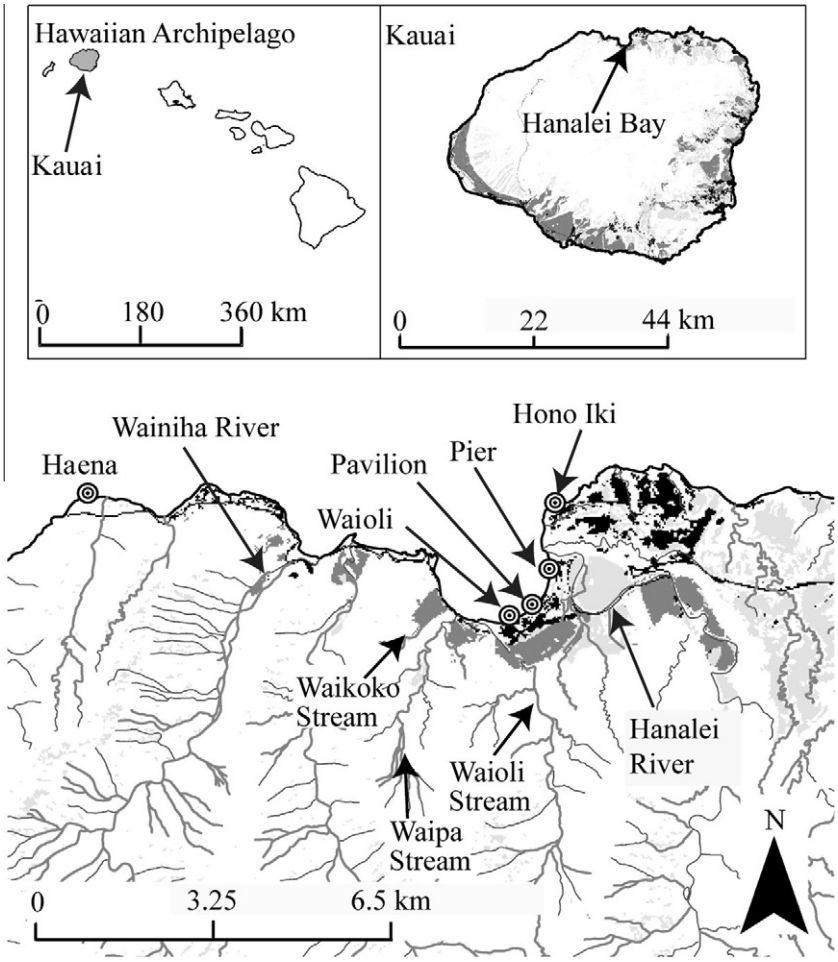

Fig. 1. Map of sites where samples were collected. Adapted with permission from Knee et al. (2008). Bulls-eye symbols indicate coastal ocean sampling sites. Gray lines on the bottom panel indicate rivers and streams. Shading indicates land use (black for urban development; dark gray for agriculture; light gray for grasslands; and white for undeveloped land, including forests, wetlands, and barren land). Land use data are from National Oceanographic and Atmospheric Administration Coastal Services Center (www.csc.noaa.gov/crs/lca/hawaii.html).

in its own right. A recent study (Pollack et al., 2009) suggested that exposure to caffeine may exacerbate the effects of other environmental stressors on corals, making them more likely to undergo bleaching.

Pesticides are another class of terrestrial pollutants that pose a threat to coral reefs and other coastal ecosystems. A previous study of the Hanalei River (Orazio et al., 2007; Fig. 1) found low waterborne concentrations of the insecticide dieldrin; however, the authors did not measure pesticide concentrations in groundwater or coastal ocean water. In the present study, we tested for carbaryl, metalaxyl, and metribuzin, three agricultural pesticides used in Hawaii (Brennan et al., 1992). These three pesticides were selected for the following reasons: (1) they have octanol-water partition coefficients indicating solubility in water, (2) publications and government reports (Kasimani, 1988; Brennan et al., 1992; Miles et al., 1992; Deputy and Hara, 2000) suggested that they might be used on the north shore of Kauai, and (3) they could be analyzed concurrently with caffeine, which was the main focus of the study.

In addition, the three pesticides we surveyed can negatively affect aquatic organisms. Carbaryl, an insecticide used on farms and lawns, can inhibit coral larval metamorphosis (Markey et al., 2007). Metalaxyl, a fungicide, is toxic to algae and zooplankton (Kungolos et al., 2009), and metribuzin, an herbicide, can harm corals' symbiotic dinoflagellate algae, leading to impaired photosynthesis and bleaching (Jones, 2005). Coral disease, perhaps related to stress from sedimentation, has been documented in Hanalei Bay (Aeby, 2007). However, no coral bleaching has been reported (Friedlander et al., 2008), and the north shore of Kauai is one of the few places in Hawaii where coral cover has actually increased over the past decade (Brown and Friedlander, 2007).

Accordingly, the primary goal of this study was to provide a preliminary survey of caffeine, carbaryl, metalaxyl, and metribuzin concentrations in groundwater and surface water at various sites on the north shore of Kauai. A secondary goal was to assess whether the pattern of caffeine concentrations we observed was consistent with a wastewater source, a natural source, or a combination of the two.

During two sampling periods in August 2006 and February 2007, a total of 61 groundwater and surface water samples were collected from five sites along the north shore of the island of Kauai, including Hàena, Hanalei Bay, and Hono Iki (Fig. 1, Table 1). The population densities and land use patterns in the vicinity of each site are reported in a previous paper (Knee et al., 2008). Four supplementary samples (two from the Hanalei River upstream of all human development and two from the offshore ocean outside of Hanalei Bay) were collected in March 2008.

Groundwater and surface water samples were collected at each site (Table 1). Groundwater samples included water flowing from coastal springs and pumped from temporary wells installed in the beach face. Temporary wells consisted of either a hand-dug pit with a screened bucket inserted in it or an augured hole with a screened PVC pipe; these wells accessed unconfined groundwater

Table 1
Caffeine detection and average concentration. Concentrations are either the value of the single sample (if $n = 1$) or the group mean value (if $n > 1$). When some samples within a group were below the detection limit for caffeine, they were assigned a value of half the detection limit (0.5 ng L^{-1}) to calculate the mean. A.d. indicates above detection limit. B.d. indicates that all samples in the group were below the detection limit. Two samples with surrogate standard recoveries below 70% were removed from analysis.

	Site	Sample type	n collected	n A.d.	Caffeine (ng L^{-1})
August 2006	Hono Iki	Temporary well	2	2	9
		Coastal ocean	1	1	10
		Puu Poa Stream mouth	1	1	28
		Queens Bath spring	1	1	8
	Pier	Temporary well	2	2	1
		Coastal ocean	1	1	9
		Hanalei River mouth	1	1	14
		Temporary well	3	3	4
	Pavilion	Coastal ocean	1	1	4
		Temporary well	3	3	12
	Waioli	Coastal ocean	1	1	7
	Hanalei	Taro field groundwater	1	1	88
	Hàena	Temporary well	1	1	17
		Coastal ocean	1	1	10
		Wainiha River mouth	1	1	25
		Small stream in State Park (mouth)	1	1	16
February 2007	Hono Iki	Temporary well	3	0	b.d.
		Coastal ocean	2	1	2
	Pier	Temporary well	6	2	2
		Coastal ocean	2	2	6
		Hanalei River mouth	2	0	b.d.
		Temporary well	5	1	3
	Pavilion	Coastal ocean	2	1	2
		Temporary well	3	2	3
	Waioli	Coastal ocean	2	1	4
		Waioli Stream mouth	1	0	b.d.
	Western	Waipa Stream mouth	1	0	b.d.
	Hanalei Bay	Coastal ocean near Waipa Stream	1	1	3
		Waikoko Stream mouth	1	0	b.d.
		Coastal ocean near Waikoko stream	1	1	5
	Hàena	Temporary well	3	1	5
		Coastal ocean	2	0	b.d.
		Coastal spring	2	0	b.d.
March 2008	Hanalei River	Upstream of human development	2	0	b.d.
	Hanalei Bay	Offshore	2	0	b.d.

<1 m below the water table. Surface water samples were collected from rivers, streams, and the coastal ocean. Coastal ocean samples were collected at a depth of <1 m, within 100 m of the shoreline.

Water samples were collected into 1-L amber glass bottles with Teflon-lined caps, which were sample-rinsed immediately prior to collection. The bottles were prepared as follows: soap-cleaned, rinsed five times with tap water and three times with Milli-Q water (Millipore Corporation, Billerica, MA, USA), soaked in 10% hydrochloric acid for at least 1 h, rinsed 5 times with Milli-Q water, rinsed 3 times with methanol, and finally either baked at 260 °C for 2 h or rinsed 3 times with dichloromethane to remove any organic contamination. Samples were stored on ice for up to 12 h, filtered through baked (500 °C for 4 h) 0.7 μm GF/F filters, acidified to pH 2 using sulfuric acid, and stored in the dark at 4 °C until extraction. The objective of filtration and acidification was to eliminate the possibility of microbial caffeine degradation during storage. A suite of other water quality parameters, including salinity and the concentrations of dissolved nutrients (combined nitrate and nitrite, phosphate, silica, and ammonium) and fecal indicator bacteria (total coliform, *Escherichia coli* and *Enterococcus* sp.) were measured concurrently and reported in a previous study (Knee et al., 2008).

Analytes were extracted into dichloromethane using liquid–liquid extraction. All glassware used for extraction was soap-cleaned, rinsed thoroughly with Milli-Q water, and then rinsed twice with methanol and twice with dichloromethane before each use. Each sample (~1000 mL) was poured into a clean 2-L glass separation funnel, and 50 μL of surrogate standard solution (consisting of 40 ppm d8-Naphthalene, d10-Acenapthene, d10-Phenanthrene, d12-Chrysene, and d12-Perylene in dichloromethane) was added using a glass syringe. 100 mL of dichloromethane was added to each separation funnel, which was then shaken by hand for 2 min and left to separate. Once two distinct layers had formed, the bottom layer (dichloromethane) was drained out through a glass funnel filled with sodium sulfate to remove any water suspended in the dichloromethane and into a clean glass flask. This was repeated three times for each sample, with 75 mL and 50 mL aliquots of dichloromethane used on the second and third extractions, respectively. After the third extraction, the sodium sulfate was rinsed with dichloromethane to flush out any remaining extract. Flasks were covered securely with methanol-rinsed aluminum foil and stored at room temperature for up to one week before concentrating the extracts.

A Rotavapor® rotary evaporator (Buchi Labortechnik AG, Switzerland; model RE-121) was used to concentrate the extracts. Each extract was poured into a clean 500-mL round-bottomed flask, reduced to a volume of 25-40 mL, transferred to a 50 mL pear-shaped flask, and evaporated to a volume of <2 mL. The concentrated extract was transferred to a new, dichloromethane-rinsed 2 mL amber glass vial with a Teflon cap. At each transfer, 2 rinses were preformed to make sure all the extract was transferred. Extracts were analyzed at California State University, Long Beach, using Capillary Gas Chromatography/Mass Spectrometry (GCMS, Agilent 6890 N/5973 N) equipped with a 60 m Phenomenex ZB-5, 0.25 mm internal diameter, 0.25 μm film thickness capillary column with a helium carrier velocity of 35 cm s^{-1}. A spiltless injection was used with a GC column temperature program from 45 °C to 125 °C at 20 °C min^{-1}, then ramped to 295 °C at 2.5 °C min^{-1} and held at 295 °C for 10 min. The injector and transfer line temperatures were 285 °C, the source temperature was 230 °C, and the quadrupole temperature was 150 °C. The mass selective detector (MSD) was used in the electron ionization (EI) full scan mode scanning from 45 to 500 AMU at a rate of 1.6 scans s^{-1}. Quantitation was based on a 5-point calibration curve using a linear equation forced through zero. The detection limit of the GCMS was 1 ng.

For quality assurance, several different types of blanks were prepared and analyzed. Three field blanks were collected concurrently with samples by pouring commercially available bottled water (Menehune Water Company, Hawaii, USA) into sample bottles in the field and then processing them identically to all other samples. Eight laboratory blanks, 3 spiked laboratory blanks, and 5 Rotavapor® blanks were also run. Laboratory blanks consisted of 1000 mL of Milli-Q water added directly to the separation funnel in the lab. Spiked blanks consisted of 1000 mL Milli-Q water with 1 mL of methanol containing 1 ppm each of caffeine, metribuzin, carbaryl and metalaxyl added. Rotavapor® blanks consisted of approximately 300 mL of pure dichloromethane evaporated on the Rotavapor® as with actual extracts. The goal of the Rotavapor® blanks was to ensure that glassware used in extract concentration was being cleaned adequately between samples.

None of the 3 field blanks and 5 Rotavapor® blanks contained caffeine. One of the 6 lab blanks tested positive for caffeine, and the samples extracted in the same batch as this contaminated blank ($n = 6$) were removed from analysis and are not included in the sample counts or data presented here. No blanks tested positive for any of the pesticides. The average (±standard deviation) percent recovery of caffeine in spiked blanks was 73 ± 10%.

The samples in this study were stored for as long as eight months prior to extraction, which is considerably longer than the storage times reported in previous environmental caffeine studies (Siegener and Chen, 2002; Buerge et al., 2003; Godfrey et al., 2007). To address the potential issue of caffeine degradation during storage, we conducted an 8-month-long degradation experiment as follows. Sample bottles were prepared as described previously and 1000 mL of Milli-Q water was added to each bottle. The bottles, hereafter referred to as "artificial samples", were then seeded with caffeine, acidified to pH 2 with sulfuric acid, and stored in the dark at 4 °C until extraction. Two artificial samples were extracted at 0, 2 and 4 weeks since preparation and then approximately every month thereafter. Blanks were extracted and analyzed concurrently with each pair of artificial samples. We did not investigate the potential degradation of carbaryl, metribuzin, or metalaxyl in stored water samples.

The results of the caffeine degradation experiment indicated that caffeine concentrations in water samples prepared and stored as described above would not be expected to decrease over an eight-month period. The caffeine concentration measured in artificial samples did not decrease with time (Fig. 2). The steps we took to preserve stored samples (filtering, acidification, and storage in the dark) would have prevented caffeine in environmental samples from adsorbing to particles, biodegrading, or photodegrading, thus making the field samples more analogous to the artificial samples. Moreover, the large variation in types of environmental waters studied here precluded degradation studies in all water types. However, we recognize that the physicochemical properties of artificial samples (acidified, caffeine-enriched Milli-Q water) and environmental samples are not identical.

All field samples, blanks, and artificial samples used in the degradation experiment were spiked with a solution containing d8-Napthalene, d10-Acenapthene, d10-Phenanthrene, d12-Chrysene, and d12-Perylene for the purpose of indicating the quality and variation of the extraction process. Average (±standard deviation) percent recoveries were 44 ± 18% for d8-Napthalene, 72 ± 16% for d10-Acenapthene, 90 ± 16% for d10-Phenanthrene, 88 ± 20% for d12-Chrysene and 108 ± 27% for d12-Perylene. The percent recovery of d12-Chrysene was high and well correlated with that of caffeine in spiked blanks and artificial samples ($r = 0.93$; $p < 0.01$). Two samples for which the recovery of d12-Chrysene was less than 70% were removed from analysis because these low recoveries indicated a problem in the extraction procedure. Based on the variation in percent recovery of d12-Chrysene, caffeine concentrations reported here should be interpreted as having relative uncertainties (as measured by the standard deviation) of approximately ±20%.

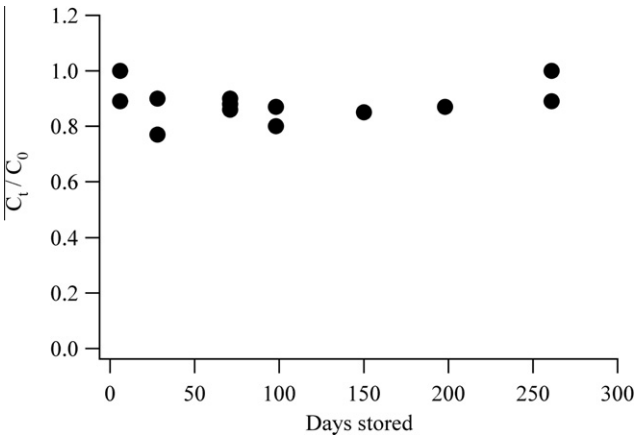

Fig. 2. Caffeine concentration after t days of storage (C_t) divided by the initial caffeine concentration (C_0) in artificial samples created for the 8-month degradation experiment. C_0 was approximated as the caffeine concentration of the first artificial sample extracted (559 ng L^{-1}; $t = 6$ days).

Because d12-Chrysene recovery in environmental samples was close to 100%, the caffeine concentrations reported here were not adjusted for percent recovery even though the percent recovery of caffeine in spiked blanks ($n = 3$) was 73 ± 10%. The caffeine concentrations reported here simply represent the mass of caffeine measured divided by the volume of the samples; thus, they may underestimate actual caffeine concentrations. We did not observe any consistent differences in d12-Chrysene recovery among Milli-Q water blanks, spiked blanks, artificial samples, and field samples. Although the high and generally consistent recovery of d12-Chrysene indicates that the extraction process was in control, we note that ^{13}C-labeled caffeine is preferable as a surrogate standard because its chemical behavior during extraction would have been identical to that of normal caffeine.

Caffeine was detected in 22 of 22 (100%) samples collected in August 2006 and 13 of 39 (33%) samples collected in February 2007 (Table 1). Concentrations ranged from below the detection limit of 1 ng L^{-1} to 88 ng L^{-1}. The caffeine concentration of treated wastewater effluent from the Princeville Utilities Company (WWTP) near Hono Iki was 39 ng L^{-1}. Caffeine was not detected in Hanalei River samples upstream of human development or in the offshore ocean.

The environmental caffeine concentrations we observed were comparable to those reported for river and well water in the south of France (Rabiet et al., 2006) and seawater in Massachusetts Bay (Siegener and Chen, 2002) and the North Sea (Weigel et al., 2002). They were somewhat lower than caffeine concentrations measured in groundwater and surface water in the southeastern United States (Peeler et al., 2006), Swiss rivers and lakes (Buerge et al., 2003, 2006), and groundwater near Reno, Nevada (Seiler et al., 1999), and more than an order of magnitude lower than maximum concentrations reported for Iowa streams (Kolpin et al., 2004), and Boston Harbor (Siegener and Chen, 2002).

Metribuzin, an herbicide used to combat numerous species of broadleaf weeds on turfgrass (Brosnan and DeFrank, 2008), including golf courses (Miles et al., 1992), in Hawaii, was detected in 5 of 39 samples in February 2007 but in none of the 22 samples collected from the same general area in August 2006. Metribuzin concentrations in coastal ocean water at Pier, Pavilion, and Hono Iki and in groundwater at Pier and Pavilion ranged from 4 to 11 ng L^{-1}. These metribuzin concentrations, which were not adjusted based on the percent recovery of d12-Chrysene, are several orders of magnitude below levels reported to be toxic to aquatic plants or algae (Forney and Davis, 1981; Jones, 2005; Pavlic et al., 2006).

Metribuzin is applied to weeds after they emerge, rather than to kill seeds before they sprout (Brosnan and DeFrank, 2008). In Hawaii's semitropical climate, weeds can emerge at any time of year (Brosnan and DeFrank, 2008), so metribuzin applications would not follow a set seasonal pattern. It is possible that the metribuzin concentrations measured during the February 2007 trip resulted from metribuzin applications to golf courses and/or lawns in the time immediately preceding the sampling trip. However, the small number ($n = 5$) of samples testing positive for metribuzin, and the fact that these samples included both groundwater and seawater from three different locations, made it difficult to draw any conclusions about the origin of the metribuzin detected in this study other than that the origin is likely terrestrial and located near the coast.

Samples were grouped by sampling trip (August 2006 or February 2007) and sample type (groundwater, coastal ocean, or river/stream), and the Student's t-test was used to test for differences between groups at the $\alpha = 0.05$ significance level. Caffeine concentrations in coastal ocean and river and stream samples were

significantly higher in August 2006 than in February 2007 (Fig. 3). A similar pattern was observed in groundwater samples, although it was not statistically significant. In August 2006, river and stream samples had significantly higher caffeine concentrations than coastal ocean samples. This pattern was not observed in February 2007, when all river and stream samples except Puu Poa Stream near Hono Iki (5 ng L^{-1}) were below the detection limit for caffeine.

Pearson correlation coefficients were calculated between caffeine concentrations and each of the following variables: salinity, nitrate + nitrite, phosphate, silica, ammonium, log *Escherichia coli*, and log *Enterococcus* concentrations (Table 2). In August 2006, significant positive correlations between caffeine and the concentrations of both phosphate and silica were observed in groundwater samples. Additionally, a significant inverse correlation was observed between caffeine and ammonium concentration in river and stream samples. In February 2007, caffeine concentration in coastal ocean samples showed a significant inverse correlation with salinity and a significant positive correlation with log *Enterococcus* concentration.

The caffeine concentrations observed in this study are consistent with a terrestrial caffeine source located near the shoreline. Samples of Hanelei River water upstream of human development and of offshore ocean water did not contain detectable levels of caffeine, while many coastal groundwater, coastal ocean, and river and stream mouth samples did. Although we did not detect caffeine in offshore or upstream samples, it is plausible that caffeine might be present in those locations under certain conditions, for example if a passing vessel discharges waste (offshore) or from caffeine-producing plants (upstream).

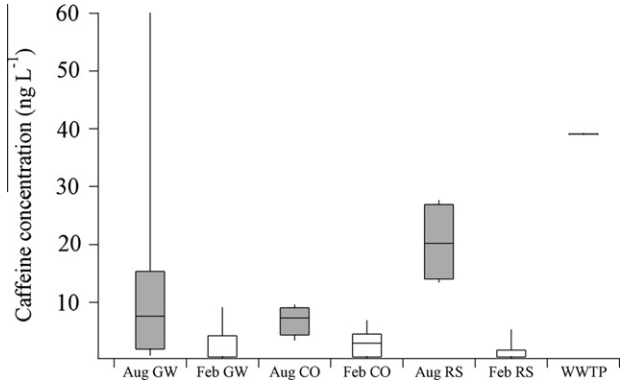

Fig. 3. Box and whiskers plot of caffeine concentrations in groundwater (GW), coastal ocean (CO), and river and stream (RS) samples in August 2006 (Aug.) and February 2007 (Feb.). The caffeine concentration of a single effluent sample from the Princeville Utilities Company wastewater treatment plant (WWTP), located near the Hono Iki sampling site, is shown for comparison as single horizontal line. Bottom, middle, and top horizontal lines in each box represent the 25th, 50th, and 75th percentile of each sample group, respectively. Lower and upper whiskers extend to the 10th and 90th percentile.

It remains unclear whether the caffeine we measured in environmental water samples originated from a natural or anthropogenic source. The significant positive correlation between caffeine and log *Enterococcus* observed in river/stream samples from February 2007 (Table 2) may suggest an anthropogenic sewage source. However, natural caffeine sources may also play a role. Caffeine is produced by over 60 plant species (Willaman, 1961; Kretschmar and Baumann, 1999), at least 14 of which grow wild or are cultivated in Hawaii (Table 3). This is in contrast to temperate and subtropical locations where previous caffeine studies have been conducted (Siegener and Chen, 2002; Buerge et al., 2003; Peeler et al., 2006), which had few or no natural caffeine sources. Except for large farms or plantations, which were not present in the study area, the distributions of these caffeine-containing plants are unknown.

Significantly higher caffeine concentrations were measured in coastal ocean and river and stream samples in August 2006 compared to February 2007. This difference did not result from differences in sample collection or preparation, since samples were collected and prepared identically during the two sampling trips. Differences in extraction procedure or percent recovery of caffeine can also be ruled out, since samples from February 2007 were extracted immediately after those from August 2006, using the same protocols and equipment, and the two batches of samples were run on the GCMS as a single group. Additionally, the consistent recovery of d12-Chrysene in samples from both trips indicated that the percent recoveries of caffeine in the two sample batches would also be the same.

The high caffeine concentrations measured in August 2006 compared to February 2007 may reflect seasonal differences in caffeine inputs, transport, or degradation. Almost 30,000 (32%) more tourists visit Kauai in August than in February (State of Hawaii, 2007) so the wastewater load would be expected to be higher in August. Differences in rainfall between the two seasons could result in differences in caffeine transport through coastal groundwater and surface water. Rainfall recorded by the United States Geological Survey Hanalei Rain Gauge in August 2006 (0.8 cm d^{-1}) was 40% lower than in February 2007 (1.3 cm d^{-1}). The greater rainfall in February 2007 and the rainy months preceding it may have diluted environmental caffeine concentrations, making them lower than in August 2006. In addition, the growth and caffeine production of caffeine-producing plants may vary seasonally in response to changes in temperature, sunlight, or rainfall.

This study showed that caffeine and the agricultural pesticide metribuzin were present in coastal groundwater and surface water samples on the north shore of Kauài. To the best of our knowledge, this is the first study to report environmental caffeine

Table 2
Pearson correlation coefficients between caffeine and other water quality variables in coastal ocean, groundwater, and river and stream samples. N + N, PO_4^{3-}, Si, and NH_4 indicate concentrations of combined nitrate and nitrite, phosphate, silica, and ammonium, respectively, in μmol L^{-1}. *Escherichia coli* and *Enterococcus* concentrations (in most probable number per 100 mL) were log-transformed to attain a normal distribution. FIB indicates fecal indicator bacteria, including *Escherichia coli* and *Enterococcus*. Asterisks (*) indicate statistically significant ($p < 0.05$) linear correlations. '–' indicates too few samples to calculate a correlation.

		n (salinity and nutrients)	Salinity	N + N	PO_4^{3-}	Si	$NH4^+$	*n* (FIB)	log *E. coli*	log *Enterococcus*
August 2006	Coastal ocean	4	−0.74	0.22	0.24	0.51	0.37	2	–	–
	Groundwater	13	−0.34	−0.20	0.83*	0.95*	0.03	13	−0.22	−0.17
	River and stream	4	−0.61	−0.25	−0.40	−0.30	−0.94*	4	0.48	0.23
	All samples	22	−0.33	−0.15	0.65*	0.74*	0.03	22	−0.09	−0.07
February 2007	Coastal ocean	9	−0.62*	0.55	0.55	0.68	0.54	9	0.34	0.64*
	Groundwater	20	0.02	0.39	−0.17	−0.23	−0.12	19	−0.09	0.41
	River and stream	6	0.90*	−0.57	−0.79	−0.81	−0.63	6	−0.69	-0.90*
	All samples	35	0.10	0.34	−0.15	−0.24	−0.14	34	−0.04	0.21

Table 3
Caffeine-producing plants found in Hawaii.

Scientific name	Common name	Details	Source
Annona cherimola	Cherimoya, custard apple	Introduced in 1790. Now casually grown and recently naturalized in upland forests	Bishop Museum; Wagner and Herbst (2003)
Camellia sinensis	Tea	Cultivated in Hawaii, mainly in Hilo/Volcano area.	Bishop Museum; Hao (2005)
Coffea arabica	Coffee	Cultivated on the islands of Hawaii, Kauai, Maui, Molokai and Oahu, especially Kona coast of Hawaii. Feral coffee is also found in abandoned fields and forests	Wagner et al. (1999)
Coffea liberica	Coffee	Similar to *C. arabica*	Wagner et al. (1999)
Cola acuminata	Cola nut	Cultivated in Hawaii	Bishop Museum
Cola nitida	Cola nut	Cultivated in Hawaii	Bishop Museum
Guazuma ulmifolia	West Indian elm	Cultivated in Hawaii	Bishop Museum
Ilex anomala	Kaawàu, àiea, Hawaiian holly	Native to Hawaii and pervasive in forests on all islands except Niihau and Kahoolawe	Wagner et al. (1999)
Ilex cassine	Holly	Cultivated in Hawaii	Bishop Museum
Ilex paraguayensis	Yerba mate	Cultivated and sparingly naturalized in some valleys	Wagner et al. (1999)
Ilex vomitoria	Yaupon holly	Cultivated in Hawaii	Bishop Museum
Theobroma cacao	Cacao	Introduced in 1850. Cultivated on Hawaii since 1890, and on all islands since 2000	Bishop Museum; Bittenbender (2005)
Theobroma grandiflora	Cupuacu	Cultivated in Hawaii	Bishop Museum
Turnera ulmifolia	Yellow alder	Cultivated on main islands and naturalized in dry, disturbed areas on Kauai and Molokai	Wagner et al. (1999)

"Bishop Museum" refers to the Bishop Museum's Annotated Checklist of Cultivated Plants of Hawaii by C.T. Imada, G.W. Stapes, and D.R. Herbst, available at http://www2.bishopmuseum.org/HBS/botany/cultivatedplants. All other data sources are cited in the references.

concentrations in a tropical location. The caffeine concentrations we observed were consistent with a terrestrial caffeine source located close to the shoreline and suggestive of a sewage source. Further research is necessary to: (1) investigate whether the caffeine concentrations observed in this study resulted from wastewater pollution, (2) investigate how caffeine is transported and degraded in the environment in tropical locations, (3) understand the mechanism behind seasonal fluctuations in environmental caffeine concentrations in this area, (4) explore the use of caffeine in addition to, or instead of, wastewater tracers currently used in Hawaii (*Enterococcus* and *Clostridium perfringens*) for identifying coastal waters impaired by wastewater pollution.

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