BACKGROUND FLUORESCENCE IN GUAM'S COASTAL WATERS

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WATER AND ENVIRONMENTAL RESEARCH INSTITUTE
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UNIVERSITY OF GUAM

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by

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ABSTRACT

The study described herein determined background levels of four fluorescent dyes (optical brighteners, sodium fluorescein, eosine Y and rhodamine WT) in Guam's coastal waters. The primary objectives were to: (1) provide a baseline for future dye trace surveys in tropical karst environments; (2) make recommendations with respect to dye and sampling site selection, positive detection criteria and background correction; and (3) re-examine previous dye trace studies on Guam based on the results of this study.

Precipitation data from five rain gauges around Guam were used for correlation analysis with sampling data. Preliminary experiments were conducted to determine sampling substrate performance and optimize sampling frequency and extraction techniques. As a result, thirteen sampling sites (subtidal and intertidal springs, dissolution fractures, and perched aquifer discharge) on Guam were monitored biweekly over a 13-month period, beginning in March 2006. In addition, seawater from four nearshore coastal locations on Guam, as well as two additional springs on Saipan, was sampled for comparison. Samples were extracted using a caustic eluent composed of aqueous ammonia, potassium hydroxide, water and 2-isopropanol. This formula worked well for all dyes of interest, but produced the highest yield for fluorescein extractions.

Guam sample data revealed that optical brightener concentrations were consistently two orders of magnitude greater than either fluorescein or rhodamine. Eosine was rarely detected. Background levels in seawater accounted for nearly 40%, 90% and 25% of optical brightener, fluorescein and rhodamine levels, respectively, detected at the thirteen sampling locations. Statistical analysis showed that background levels of the four dyes varied significantly within and between sites over time. Sample data tended to correlate most strongly with data from the nearest rain gauge. Seven of eight monitoring sites exhibited an inverse correlation between rainfall and optical brightener concentrations. Fluorescein and rhodamine concentrations, on the other hand, remained remarkably stable once the wet season began. These findings suggest that surface runoff rather than submarine groundwater discharge exerts the greatest influence on background levels of fluorescence. Accurate detection of dyes is hampered during the dry season, and by background levels in the surrounding seawater. Recommendations for future dye trace studies are presented and discussed.

INTRODUCTION

Karst aquifers -- a special type of carbonate aquifer comprising approximately 25% of Earth's and 40% of the United States' groundwater resources (Green *et al*., 2006) -- are particularly susceptible to contamination. In contrast to more common porous media, karst aquifers exhibit amplified hydraulic responses due to their rapid recharge and high permeability. Dye trace studies provide a general description of the paths and average linear velocity of water movement through an aquifer from points of recharge to points of discharge. Such knowledge in turn provides insight into potential sources and pathways of contaminants, as well as discharge zones that are vulnerable to contamination.

Past surveys conducted on Guam have indicated that the Northern Guam Lens Aquifer (NGLA) is an archetypal island karst aquifer which exhibits triple porosity (Jocson *et al.,* 2002) and possesses an anisotropic and heterogeneous matrix comprised of highly permeable, eogenetic limestone (Mylroie and Vacher, 1999). Consequently, contaminants released into the water table at vulnerable locations can migrate faster through more direct conduits and discharge into the recreational waters of Agana and Tumon Bays within days or even hours (Moran and Jenson, 2004). Past dye trace studies (AAFBER, 1995; Moran and Jenson, 2004) have also shown that dyes -- and potentially, therefore, other chemicals -- introduced into wells and sinkholes located in the interior of the island (which penetrate the vadose zone and connect directly to the water table) can follow diffuse flow pathways and reside within the aquifer matrix for years.

Tracing the path of groundwater flow with fluorescent organic dyes has become a core technique in hydrogeology (Smart and Karunaratne, 2002). In organic-rich environments, however, fluorescent organic compounds are rarely used for groundwater tracing due to their affinity for adsorbing onto organic particles in the matrix, as well as their spectra being camouflaged by those of natural organic substances (Otz *et al.,* 2004). Xanthene dyes and optical brighteners are common tracers (Aley, 1999; Flury and Wai, 2003). Xanthene dyes fluoresce in the green to red portion of the spectrum, and include sodium fluorescein (trade name Uranine), eosine (specifically eosine Y, sometimes also called eosin), and variants of rhodamine (mainly rhodamine WT and sulforhodamine B). Optical brighteners, also referred to as fluorescent whitening agents, are less commonly used and are a poor choice in many situations as they are ubiquitous in the environment and fluoresce in the same range as a host of organic compounds, such as fulvic acids (Käss, 1998).

Injected dyes are not the only materials in groundwater and coastal waters that fluoresce. Many pollutants and natural compounds fluoresce as well, and can interfere with the accurate interpretation of dye trace results (Moran and Jenson, 2004). According to Smart and Karunaratne (2002), the most important criterion for assessment of a dye trace is whether the tracer used can be demonstrated to "significantly exceed background concentrations". Therefore, in order to effectively interpret the results from a dye trace study in tropical karst environments like northern Guam, it is important to understand the spatial and temporal variations of background 'noise' (*i.e.*, naturally occurring fluorescence) associated with sampling locations.

It is essential to have as complete an understanding of the background noise associated with groundwater discharge samples as possible in order to select appropriate dyes for dye trace studies and to confidently interpret the signatures and intensities of selected dyes against background 'noise'. In any dye trace study, the researcher typically obtains and analyzes background samples – samples collected from each sampling location prior to injection of the dye – in order to subtract those levels from future sample analysis results. Background levels may reflect a consistent signal or random noise, and can occur at any point along the sampling analytical trail. However, so few background samples collected do not offer high confidence in the interpretation of trace data. In addition, the nature and sources of environmental background noise are often obscure and require strategic sampling protocols in order to reduce or eliminate them (Smart and Karunaratne, 2002).

Objectives

The objectives of this project were to: (1) provide a baseline for future dye trace surveys in tropical karst environments; (2) investigate patterns and potential sources of variation in background fluorescence; (3) make recommendations with respect to dye and sampling site selection, positive detection criteria, and background correction in tropical karst environments; and (4) re-examine previous dye trace studies on northern Guam (Moran, 2002; Moran and Jenson, 2004; Earth Tech, 2006[draft]) based on the results of this background study.

To the best of my knowledge, this study is the first of its kind. Little to no literature exists concerning the spatial and temporal variation of background levels in any waters, much less submarine groundwater discharge in the tropics. The most likely sources for related background studies would come from Florida or the Bahamas, where the hydrogeology is similar in many respects to Guam's. Although requests for research related to this project were issued to various organizations (*e.g.*, water management districts, universities and environmental protection agencies) in Florida, no relevant information has been received as of this writing.

Physical Setting

Geography

Guam is the largest and southernmost of the Mariana Islands (Figure 1). It is located between 13º26' and 13º35' East longitude and 144º41' and 144º52' North latitude. Roughly 549 square kilometers in area, the island is divided by a major fault into two distinct physiographic provinces (Taboroši *et al.,* 2004): the northern half, which is characterized by a broad, limestone plateau that slopes inland from the coast; and the southern half, which is dominated by heavily weathered volcanic rock dissected by faults and fractures and fringed with limestone (Tracey *et al.,* 1964).

Hydrogeology

Two limestone formations -- the Mariana and the Barrigada -- comprise the Northern Guam Lens Aquifer (NGLA), the primary source of drinking water for the island (Barner, 1995). The Mariana Limestone is a shallow-water reef deposit comprising the cliffs of the northern plateau, which slopes gently toward the southeast. It is underlain by the Barrigada Limestone, a formation of deeper water lagoonal deposits and the dominant unit of the Northern Guam Lens Aquifer (Figures 2 and 3).

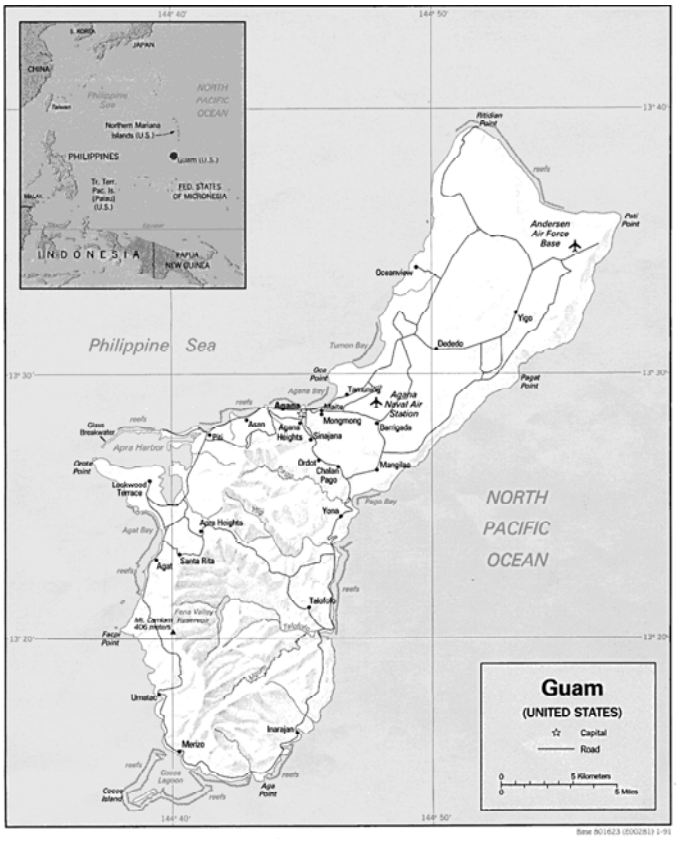

Figure 1. Map of Guam showing geographic location. Map Source: Wikipedia.

Eogenetic karst aquifers in Pliocene-Pleistocene limestone such as the NGLA typically exhibit secondary porosity consisting of connecting voids and conduits zigzagging through a permeable matrix of interparticle porosity (Vacher and Mylroie, 2002). Eogenetic karst is spatially and temporally located close to its depositional environment, and usually occurs at low latitudes. Florea and Vacher (2007) showed that spring hydrographs from Florida's eogenetic karst environment are not "flashy"; rather, they have smooth, extended seasonal maxima. Hydrographs from both cave and non-cave conduit systems indicate direct connectivity between the permeable matrix and secondary porosity structure.

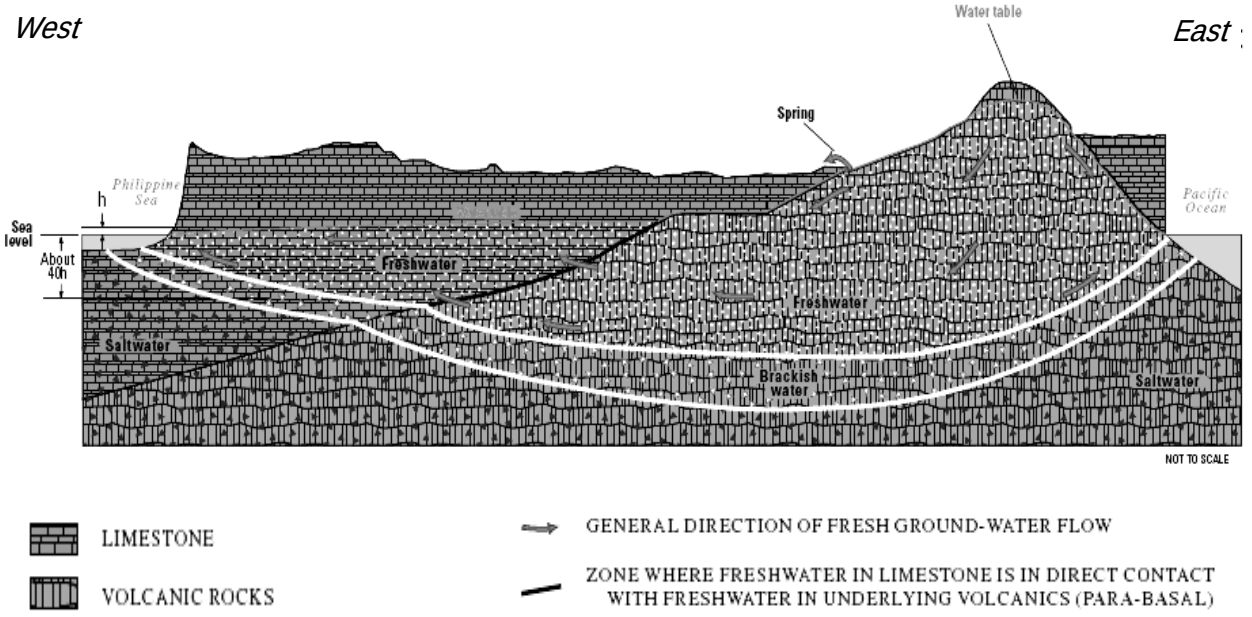

Figure 2. Generalized groundwater occurrence on northern Guam. Source: USGS.

A review of geologic surveys that have been conducted to substantiate preceding mapping efforts of sinks and faults (PIE, 1950; Tracey *et al.,* 1964; and Siegrist *et al.,* 1998) revealed a series of fault scarps in the Jonestown and Tamuning areas. In addition to these features, the Geologic Map of Guam (Figure 3, from USGS, 2003) portrays several sinkholes lying west of Harmon Sink along an east-southeast to west-northwest axis, suggesting a relationship with fracture orientation.

Climate

The westward-moving, warm, humid air typical of tropical latitudes determines the climate of Guam. Mean monthly temperatures vary little, ranging from ~ 25 to 26° F, while mean monthly precipitation values vary widely, ranging from as low as 7 cm in the dry season (December through May) to as high as 36 cm in the wet season (June through November) (NWS, 2007). This semi-annual seasonality of rainfall may have an influence on the volumetric discharge of residual dyes from the aquifer.

The El Niño Southern Oscillation (ENSO) contributes to the variability of rainfall on Guam from year to year (Lander *et al*., 2001). The NGLA is fairly responsive to intense rainfall events, although periods of prolonged drought occur the year following El Niño. During this time, the aquifer does not respond

appreciably to the first heavy rain events which break the drought (Lander *et al*., 2001). In a geologically similar environment (north-central Florida), Florea and Vacher (2007) found that hurricane events raised the water table considerably and quickly, while normal summer rain events did not.

Related Previous Research

Little to no research exists concerning the spatial and temporal variation of background levels in any waters, much less submarine groundwater discharge in the tropics. Background samples collected in previous dye trace studies (AAFBER, 1995; Moran and Jenson, 2004; Earth Tech, 2006[draft]) on Guam (1) demonstrate the inadequacy and variability of background concentrations, and (2) resolve positive dye detections based on overly conservative criteria.

Hydrogeologic Studies on Guam

Mink and Vacher (1997) summarized the results of previous hydrogeologic studies of the NGLA. Spring location inventories and studies concerning relationships between groundwater discharge rates, water chemistry, water quality, and seasonality have been performed along the northwestern coast of Guam (Ayers, 1981; Matson, 1993; Jenson *et al*., 1997; Jocson *et al.,* 1999; Contractor and Jenson, 1999; Taboroši *et al*., 2004). Moran and Jenson (2004) summarized dye trace studies conducted at a landfill closure site on Andersen Air Force Base and at a U.S. Navy housing complex in Finegayan in the 1990s. These studies showed that: (1) groundwater flows through the NGLA by diffuse, gradient-driven flow towards Agana and Tumon at rates of 10^2 to 10^3 m/day; (2) perpendicular flow paths are consistent with regional fracture orientation; and (3) rapid, discrete conduit flow occurs within the epikarst and lower vadose zones of the aquifer. Results from all of these studies illustrate the complexities of carbonate island karst aquifer systems.

Dye Trace Studies on Guam

Although several dye traces have been performed on Guam over the last few decades, only three were located that included data pertaining to background sampling: an investigation of Harmon Sink (Moran, 2002; Moran and Jenson, 2004); and two abandoned military landfills, one for the Air Force in 1992 (AAFBER, 1995) and one for the Navy in 2004 (Earth Tech, 2006[draft]).

With respect to background fluorescence, each of these studies had particular limitations and inadequacies, discussed in detail in a later chapter, including the number of background samples collected, time of year collected, and inconsistent detection criteria. A weakness shared by all three studies was insufficient volumes of eosine used. Eosine Y requires a concentration of over three times that of fluorescein in order to obtain similar intensities during analysis (by comparison, rhodamine WT requires an excess of 25 times the amount of fluorescein). Using fluorometric analysis, eosine can only be detected down to parts per billion (ppb), as opposed to parts per trillion (ppt) for fluorescein. Not surprisingly, eosine was rarely, if ever, detected in these studies.

Other Studies

In 2002, Smart and Karunaratne conducted a long-term, baseline study on levels of fluorescent materials in Canadian surface waters. In this study, daily grab samples were collected over a six month period from a gauging station on Medway Creek in Ontario, a surficial system which drains a mix of agricultural and suburban catchments over glacial till. Interference was reported in the range of fluorescein, and the composition of general organic background noise exhibited considerable temporal variation. Intensities at various wavelengths seemed to fluctuate in response to stream discharge. The authors suggested that karst aquifers in analogous environments would yield similar results.

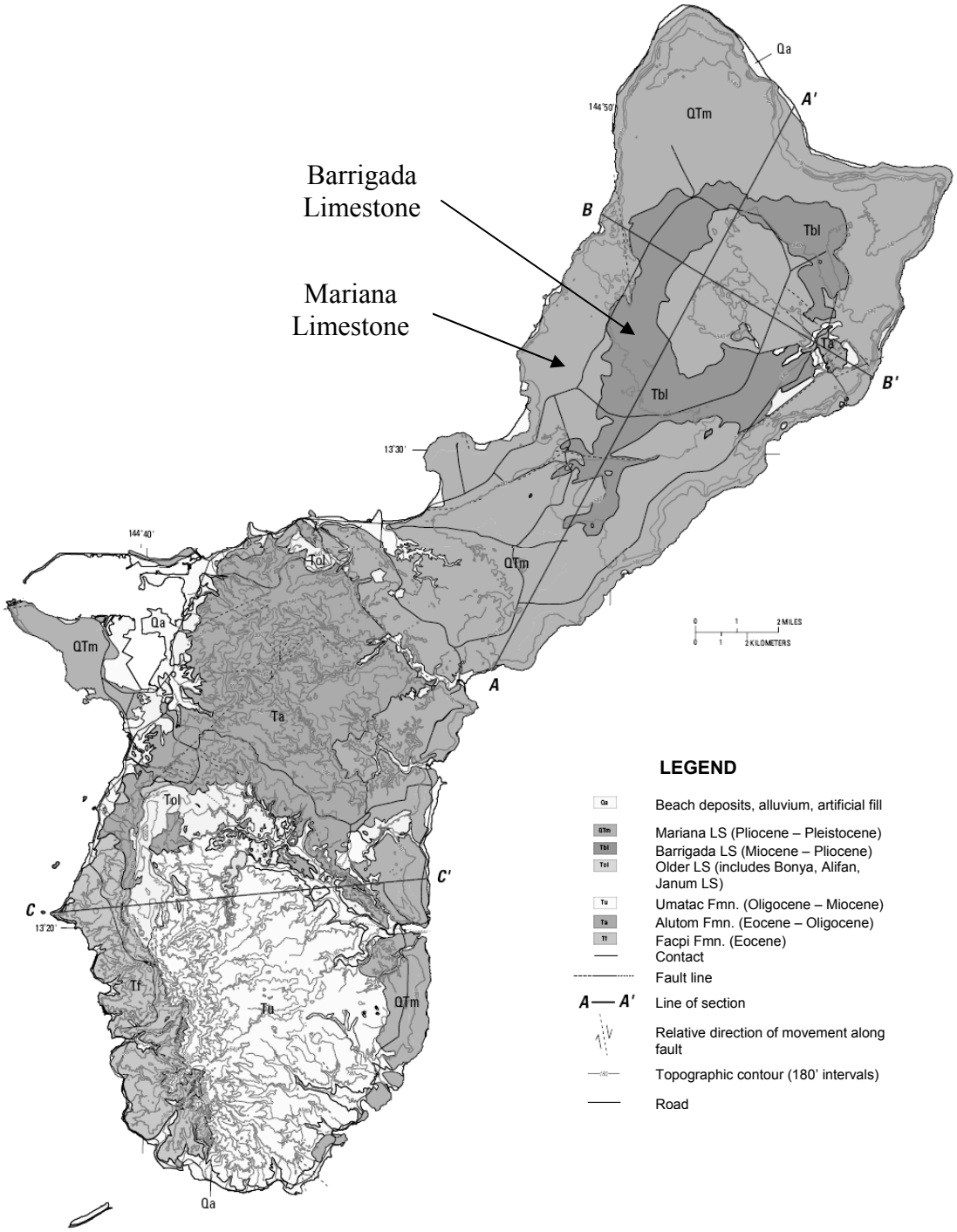

Figure 3. Geologic map of Guam. Source: USGS.

MATERIALS AND METHODS

SAMPLING SUBSTRATE PERFORMANCE EXPERIMENTS

One of the objectives of this project was to examine potential sources of variation in fluorescence detected in natural waters. Therefore, a laboratory study was designed to investigate the behavior of the sampling substrate (*i.e.*, granular, activated charcoal, or GAC) used in the field. The goal of this experiment was the optimization of sampling frequency based on adsorption kinetics. This was attained by determining (1) the responsiveness (*i.e.*, adsorption rate) of GAC to pulses of dye which pass over it in the field, and (2) the integrity of the bond once adsorbed (*i.e.*, desorption rate and extraction efficiency). The former was accomplished by analyzing GAC that had been soaked in high and low concentrations of dye in water for progressively longer intervals of time, thereby obtaining adsorption rates. The latter was achieved by analyzing GAC which had been saturated with dye and then placed in clean water for increasing time intervals, thereby obtaining desorption rates. A full description of this series of experiments is given by Hoffman (2006). It was assumed that GAC would be highly responsive and stable, as evidenced by the widespread use of GAC in dye tracing. It was also expected that all samplers would reach equilibrium at similar rates during adsorption.

Properties of Granular Activated Carbon

Organic compounds have a greater affinity for solid, organic substrates (e.g., GAC or river sediments) than the water in which they are suspended. Adsorbed molecules are held onto GAC surfaces by Van der Waal's forces and capillary action (Namane *et al.*, 2005). GAC is ideal for field sampling. Not only is it inexpensive and easy to use, but it is also an optimal substrate for adsorption of fluorescent materials in water, possessing a massive amount of surface area (Aley, 1999).

Prefabricated mesh packets containing GAC created via thermal treatment of coconut husks were obtained from Ozark Underground Laboratory (OUL) in Protem, Missouri in 2005. According to the supplier, each gram of GAC has a surface area of nearly 1000 m^2, while the granules have an average diameter of 5 mm, an average pore diameter of 2.6×10^{-6} mm, and an average density of 0.45 g/cm^2 (Aley, 1999).

Results

Ten packets of GAC were removed from the lot and sieved to confirm the distribution of grain sizes. The grains were tabular, so accurate sieving was difficult. Sieves of the following increments (in millimeters) were used: 4.699, 2.000, 1.000, 0.710 and 0.417. The results were conspicuously uniform, and considerably lower than the supplier's claim. Just over 95% of the grains were between 2.000 and 4.699 mm in diameter; the rest were between 1.000 and 1.999 mm. The average mass of charcoal per sampler was 5.68±0.35 g. Furthermore, an analysis of the mass of GAC lost during deployment was conducted. Twelve bugs were selected at random to be reweighed after drying. The data indicated that an average of 6.9% of the mass was lost during deployment.

Adsorption Experiment

The data indicated a rapid response to environmental changes in dye concentration, supporting the assumption that GAC is highly responsive in the environment. Within two hours, most of the samplers had reached their final maximum concentration. Forward linear modeling indicated that the samplers would not reach equilibrium at similar concentrations, which varied depending on the dye and the concentration to which the GAC was originally exposed. This finding is not surprising. Given each dyes' octanol-water partition coefficient -- log K_{ow} of -0.39, -1.33 and -1.33, for fluorescein, eosine and

rhodamine, respectively (Field *et al*., 1995) -- each dye has a low soil-water partition coefficient (K_{oc} <1). This means they are highly soluble (*i.e.*, highly mobile in the environment) and will constantly partition to a small degree between the charcoal substrate and the aquatic environment. Therefore, the more dye in the environment, the more dye that will adsorb onto the charcoal, and vice versa.

Desorption Experiment

Results from the desorption study confirmed the preconception that GAC would effectively capture dye. After deployment of dye-soaked samplers in dechlorinated tap water, it was expected that dye concentrations in the samplers would either decrease or show no change. All three dyes behaved accordingly and did not appear to desorb from the substrate. GAC concentrations remained fairly stable. Conversely, it was expected that concentrations of dye in the water would either increase (indicating loss from GAC) or remain unchanged. Water levels also remained relatively stable over time. As such, variability associated with losses from the GAC was determined to be minimal.

Extraction Efficiency Experiment

Finally, the extraction efficiency experiment revealed two noteworthy considerations. First, not all adsorbed dye is removed from GAC even after four elutions, much less a single elution. Second, the eluent used in this project, and many other dye trace studies, was most effective on fluorescein (nearly 75% yield after the first elution), and only moderately effective on the other two xanthene dyes (less than 50% yield).

Conclusions

Based on these results, it was concluded that bugs deployed in the field are highly responsive, recording virtually all "pockets" of dye which pass over them fairly quickly. Furthermore, for bugs collected biweekly or monthly, desorption during deployment does not appear to be a major concern during field sampling. Furthermore, with respect to solvents, one size does not fit all. Detections of low concentrations of eosine and rhodamine are potentially being missed during dye trace studies in which only one solvent is used for elution.

FIELD STUDY

To accomplish the project's objectives, a review of literature and available maps (*i.e.*, satellite imagery and topographic maps) was conducted. Precipitation data from selected rain gauges were collected for correlation with sample data. Sample data was obtained via field sampling and laboratory analysis using spectrofluorometry.

Precipitation Records

Five rain gauges in the vicinity of the sampling sites (Figure 4) were chosen for correlation analysis with field data. Data for the east Agana Bay sites were taken from a gauge located in Oka Point in the Tamuning area, available online through PCR Environmental. Data for the Tumon Bay sites were taken from a rain gauge at the municipal airport in Tiyan, available online through the National Weather Service. Data for the Togcha Bay site were collected from a gauge in Ipan, available online through Jeff's Pirates Cove. Data for the Pago Bay site was provided courtesy of Guam Community College in Mangilao. Data for the Asma Fenas River site, provided courtesy of the Water and Environmental Research Institute at the University of Guam, was collected from a rain gauge located at the crest of the Ugum watershed atop Mount LamLam, the highest point on Guam.

Field Sampling

Thirteen freshwater discharge locations were monitored on Guam between February 2006 and April 2007 (Figure 5, Table 1). Of these, 10 sites were located on the west coast: two in East Agana Bay, five in Tumon Bay, and three farther north near Double Reef. Two more were located on the east coast, one each in Pago and Togcha Bays. The last site was a contact spring on the flanks of Mount LamLam. The western sites were selected to represent discharge sites commonly sampled during dye trace studies, all of which are sourced in the NGLA. The two eastern sites were added due to the potential influence of specific environmental factors (*e.g.*, landfill leachate and wastewater effluent). The LamLam site was chosen as a pristine aquifer for experimental control. Additional control samples were collected from seawater at four reef flats: Pago, Dadi, Luminao, and Paseo. Also, a few samples were collected for comparison from two sites in Saipan during the spring and summer of 2006.

The frequency of sampling was determined from prior analysis of preliminary grab samples collected at sites in Tumon and Agana Bays. Relative sample intensities indicated that it was necessary to leave samplers deployed for many days to adsorb sufficient fluorescent materials to be detected. Samplers were originally intended to be left *in situ* for approximately two weeks at a time. This interval varied, however, according to sampling opportunities and field conditions. Sample receptors consisted of 2" x 4" fiberglass mesh bags containing GAC (hereafter referred to as "bugs). Bugs were placed in pairs at each sampling location and analyzed separately to estimate variability between them.

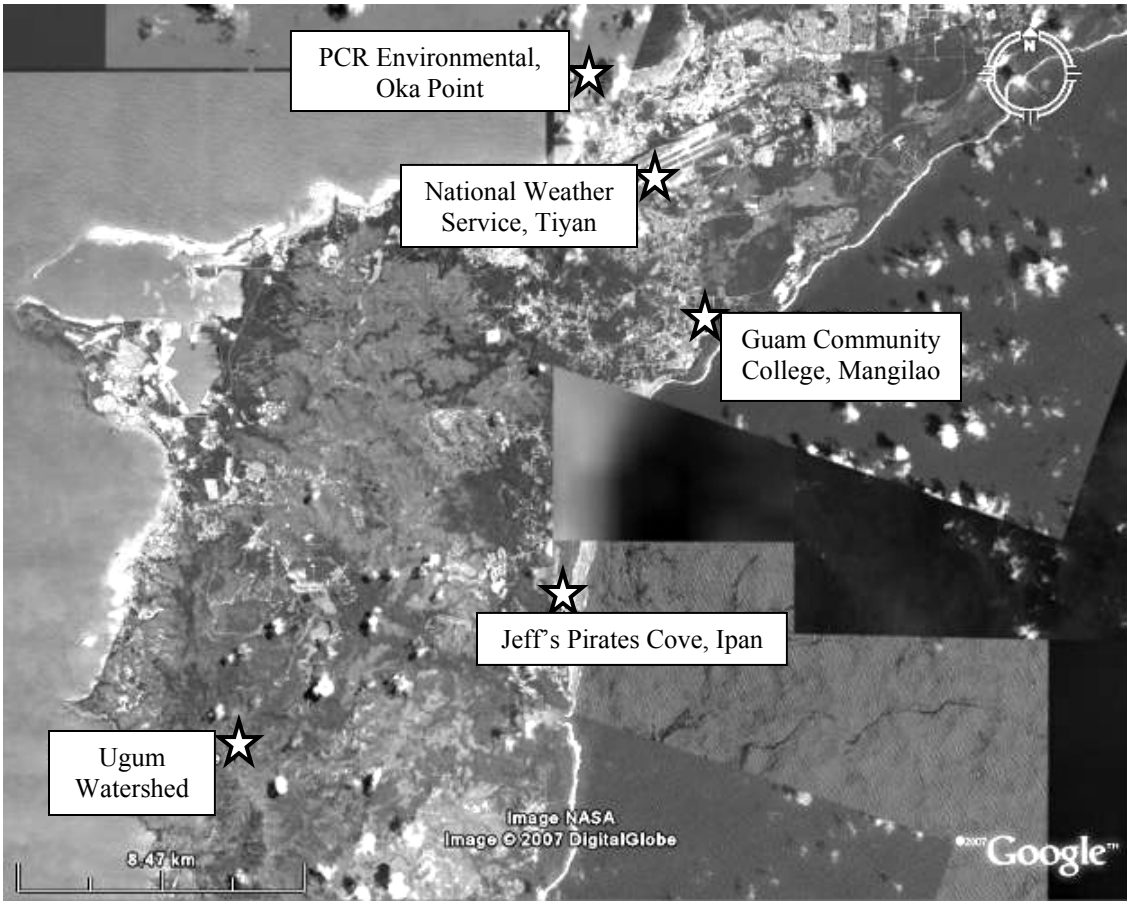

Figure 4. Locations of five rain gauge stations located in the vicinity of sampling sites. Map source: Google Earth, 2007.

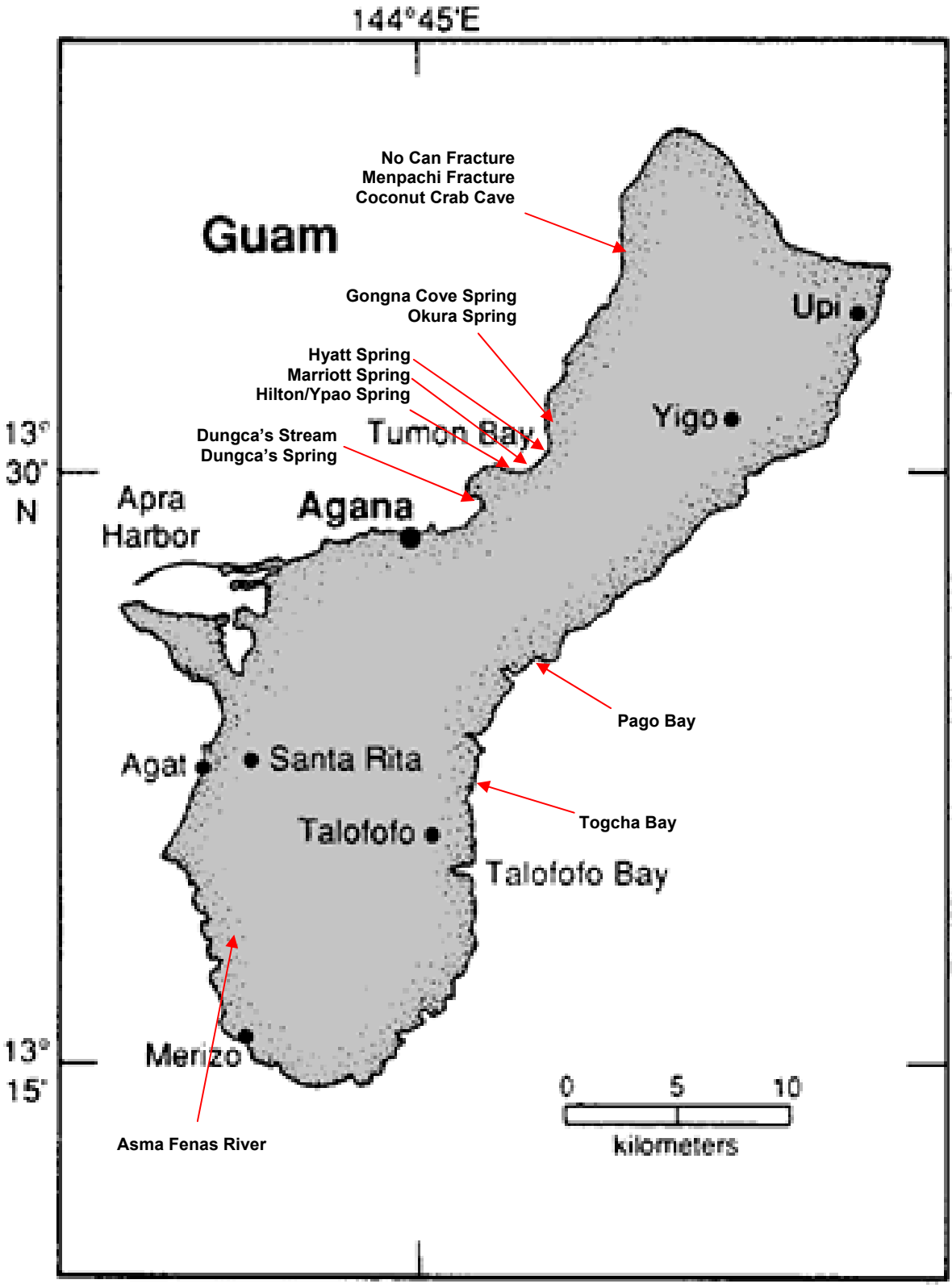

Figure 5. **Sampling locations on Guam. Map source: Pacific Business Center Program website (University of Hawai'i).**

Table 1. Sampling site names, locations and start/end dates

SITE NAME	Lat. N	Long. E	Sampling Began	Sampling Ended
ON GUAM:				
Northwest Coast				
Coconut Crab Cave	13.586	144.834	04/28/2006	05/23/2006
No Can Fracture	13.601	144.837	04/28/2006	05/23/2006
Menpachi Fracture	13.591	144.837	04/28/2006	02/12/2007
Tumon Bay				
Ypao Beach Spring	13.505	144.787	03/04/2006	04/05/2007
Hyatt Spring	13.513	144.803	03/04/2006	04/05/2007
Marriott Spring	13.506	144.795	03/04/2006	10/31/2006
Okura Spring	13.520	144.806	03/04/2006	04/05/2007
Gongna Cove Spring	13.521	144.806	10/31/2006	04/05/2007
Agana Bay				
Dungca's Stream	13.487	144.775	02/20/2006	04/05/2007
Dungca's Spring	13.488	144.774	03/04/2006	04/05/2007
East Coast				
Pago Bay Spring	13.419	144.784	04/23/2006	03/24/2007
Togcha Bay Spring	13.368	144.770	04/23/2006	03/18/2007
Control Sites				
Asma Fenas River	13.336	144.653	05/04/2006	12/15/2006
Pago Reef Flat			05/04/2007	07/04/2007
Dadi Beach Reef Flat			05/04/2007	07/04/2007
Luminao Reef Flat			05/04/2007	07/04/2007
Paseo Reef Flat			05/04/2007	07/04/2007
ON SAIPAN:				
Bird Island Beach Spring	15.260	145.813	04/14/2006	12/02/2006
Beach Road Spring	15.199	145.717	04/14/2006	07/21/2006

NOTE: GPS locations obtained using Garmin ForeTrex handheld receiver & verified using Google Earth

The construction of samplers varied between sites. Samplers in Tumon and Pago Bays were fashioned using concrete blocks broken in half to produce a U-shaped housing for the bugs, which were then attached to a braided, nylon rope tied around the block using plastic "zip-ties". The sampler in Togcha Bay was made with a large fragment of coral, instead of a concrete block, for added camouflage and a better fit inside the spring vent. Samplers in Agana Bay were tied to ropes, one which anchored a buoy in a spring vent and the other which was strung across a stream. Samplers used at the northwest coastal locations utilized key-hole fissures in adjacent rock formations as anchoring points. Grab samples of spring water discharge and surrounding seawater were randomly collected using 20-mL glass vials from each sampling location to record pH and salinity, as well as for fluorometric analysis. Grab samples were also sometimes collected in place of missing samplers.

Fluorometric data were collected from laboratory analyses of grab water samples and GAC from field samplers from the following points of discharge: (1) vertical dissolution fractures and a flank margin cave located on the northwest coast of Guam; and (2) subtidal and intertidal groundwater discharge points located along the west coast in Tumon and Agana Bays, and along the east coast in Pago and Togcha Bays. On Guam, as in most coastal and island karst aquifers, the majority of discharge points are along the coast. Exceptions to this include inland discharge points, such as rivers or artesian springs, from perched and confined aquifers.

Data were also obtained from control and comparison samples collected. A surface stream that discharges from a limestone aquifer perched atop the volcaniclastic flanks of Mount LamLam was chosen as a control site due to the absence of anthropogenic influences above the stream. Thirty-one grab samples and eight GAC samples were collected from nearshore seawater to account for natural background levels in the waters surrounding discharge points. In addition, personal visits to Saipan during the spring and summer of 2006 allowed the opportunity to collect GAC samples for comparison.

During each sampling round, intact pairs of bugs were collected and replaced with a fresh set. Based on recommendations by Käss (1998), the bugs were immersed in their respective sampling waters prior to deployment and kneaded lightly so that all grains were equally moistened and carbon dust and air bubbles were eliminated. Upon collection, the bugs were kneaded and rinsed vigorously in their sampling waters in an attempt to remove biofilm, tiny biota, and sediment. The bugs were then wrapped in aluminum foil to prevent cross-contamination and protect adsorbed fluorescent materials from photodegradation. Wrapped samples were placed in resealable plastic storage bags, labeled using permanent markers to identify sampling site and collection date, placed in a clean cooler and kept chilled with laboratory cold packs. When grab samples were collected, a trip blank was included and stored with field samples collected. Trip blanks were analyzed with field samples to account for any contamination occurring during that particular sampling round.

Description of Sampling Sites

Along the western coast of northern Guam, freshwater discharges in a variety of ways: flowing fractures and caves in coastal cliff faces; springs and seeps along beaches, platforms and reefs; and submarine spring vents (Jenson *et al*., 1997). Most of the island's coastal discharge occurs along the northwestern coast from Agana Bay to north of Double Reef, although a few springs are also located along the eastern coast. Taboroši *et al*. (2004) noted that discharge points tend to be concentrated along the northwestern coast as a result of the relative shapes and sizes of their watersheds, in addition to the underlying geology.

A detailed survey of the coastal springs and seeps was conducted to identify discharge features of interest in Agana, Tumon, Pago and Togcha Bays, as well as along the northwestern coast. Although the field names used in this study reflect adjacent landmarks, all locations were neutrally identified by their GPS coordinates (Table 1). Sampling locations around Guam and Saipan are shown in Figures 6 through 11.

Dissolution Fractures and Flank Margin Caves

Conduits feeding coastal springs are more readily seen along rocky shorelines and coastal cliffs, where fresh water can be seen flowing from fractures in the rock. Such fractures occur in a wide range of scales, from centimeters to meters wide, dissolutionally widened over time by the water that flows through them. When snorkeling up to the largest fractures, fresh water discharging from these fractures can be observed as a clear, cool layer floating on the underlying seawater; when disturbed, a cloudy mixing zone can be seen (Taboroši, 2004). Three of such sites along the northwestern coast of Guam were chosen for this project (Figure 6). No Can Fracture and Menpachi Fracture are vertical dissolution features, and Coconut Crab Cave is a flank margin cave.

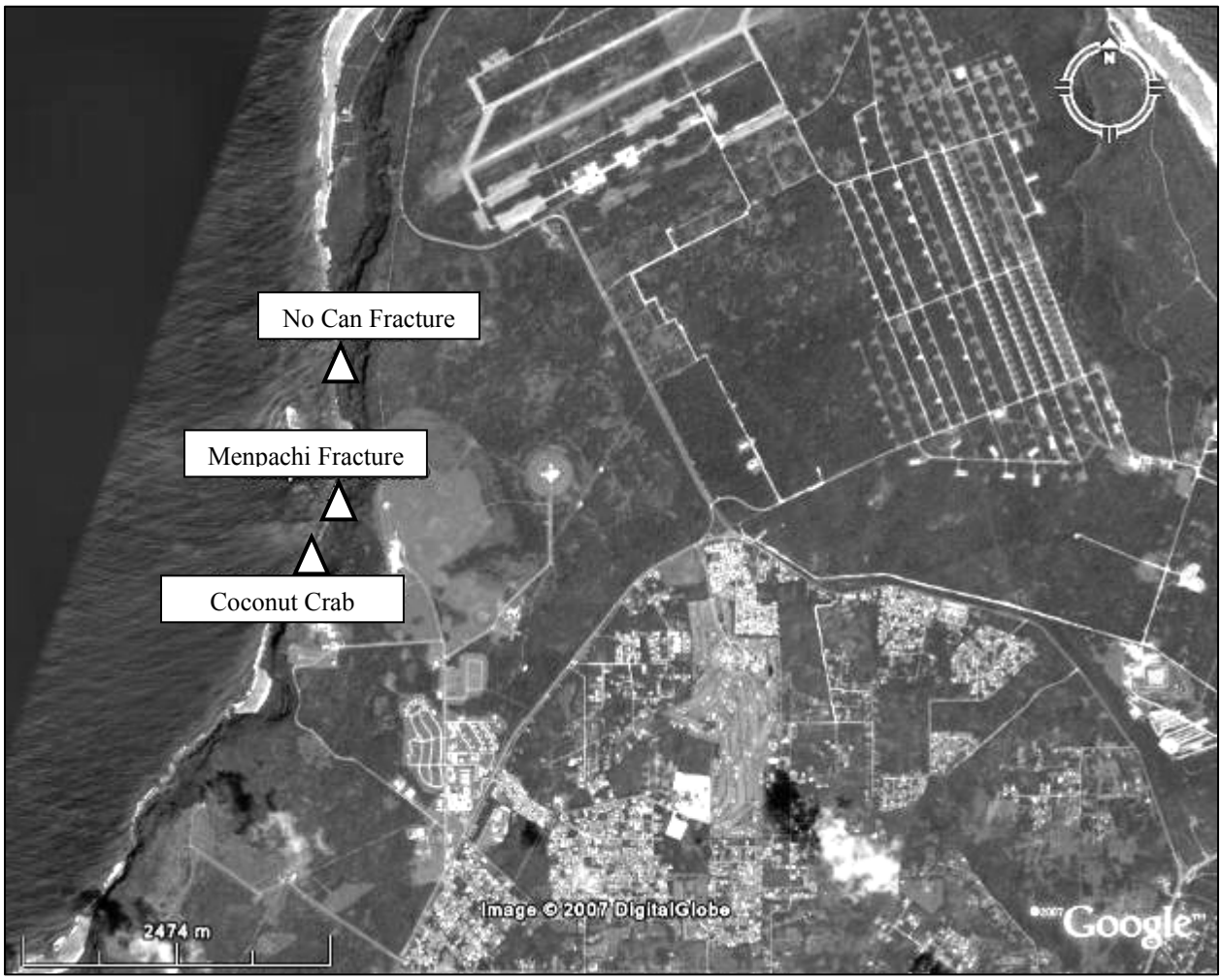

Figure 6. Satellite image of northern Guam sampling locations. Map source: Google Earth, 2007.

No Can Fracture

The longest explored discharging fracture on Guam, No Can was surveyed and described in November 1999 (Taboroši, 2004). Located approximately 1 km north of Double Reef, No Can Fracture was the northernmost sampling location for this project. This fracture receives fresh water discharge from the northern limestone plateau underlying Andersen Air Force Base (AAFB). Like other dissolution

fractures located in the coastal bench north of Double Reef, No Can Fracture discharges millions of liters of freshwater per day (Jocson, 1998; Jocson *et al.,* 1999).

The entrance to No Can Fracture is tight, and obstructed by several large, wedged-in boulders. A braided, nylon rope was tied around the rear end of one of the boulders that is continually submerged throughout the tidal cycle, toward the fracture's interior, away from the open sea. To shelter them from sunlight and wave action, bugs were attached to the rope on the underside of the rock using plastic "zip ties". Subsequently, it was not necessary to enter the fracture; the samplers could be accessed from the coastal shelf along the entrance to the fracture, reducing the danger of injury due to incoming swells and tidal surge.

Menpachi Fracture

Menpachi Fracture was mapped and described in March 2000 (Taboroši, 2004). The fracture is a very wide dissolution feature. It is characterized by tributary fractures, which contribute to its total discharge, and dissolution scalloping along its walls, which was created by water flowing through the fracture. Menpachi Fracture is located roughly 300 m north of the beach at Double Reef.

Like No Can Fracture, Menpachi receives fresh water discharge from the portion of the NGLA underlying AAFB. It is open to the ground surface above, unlike No Can Fracture which has a rock ceiling. Since Menpachi Fracture is much wider and easier to navigate than No Can Fracture, samplers were placed deeper inside, approximately 10 m from the mouth of the fracture. A 'keyhole' in the rock wall roughly 30 cm below mean sea level provided a point to which a braided, nylon rope could be tied, for the purposes of attaching bugs with zip ties.

Coconut Crab (Ayuyu) Cave

Surveyed and described in October 1999, Coconut Crab Cave is the single largest coastal spring in northern Guam. The entrance to this breached flank margin cave is located about 300 m south of the beach at Double Reef in a small cove with several large boulders at the mouth – possibly a collapsed cave room. A steady stream of fresh water flows from the cave at the waterline, estimated at 1.9×10^3 m^3/day (Taboroši, 2004). Fresh water discharges from a spring located in the northern corner of the cave entrance. A braided, nylon rope was tied through a 'keyhole' in the rock wall where it is continually submerged and protected from direct sunlight, to which bugs were attached with "zip ties".

Subtidal and Intertidal Spring and Seep Locations

Springs are distinguished from seeps by the presence of distinct vents from which fresh water discharges; seeps are not so focused, and are usually observed only at low tide, when the flow forms small channels and deltas in the sand along the beach (Taboroši *et al.,* 2004). Larger springs, such as Dungca's Spring in Agana, produce "boils" that are visible even at high tide or in breezy conditions as smooth, elliptical patterns on the water's surface.

A total of seven sites were monitored in Agana and Tumon Bays (Figure 7). These bays are very similar in their morphology, and both are major tourist centers for the island of Guam. The coastal waters in these areas are therefore of significant economic importance. A primary concern is the subsurface transport of environmental contaminants originating from neighboring commercial and industrial properties into the bays.

Tumon Bay

Tumon Bay extends to the northeast from the Hilton Hotel, due north past the Hyatt Hotel, and swings back to the northwest ending at the Nikko Hotel. Four shoreline sites were initially chosen in Tumon Bay for sampling: Ypao Beach and the Hyatt, Marriott, and Okura hotels. The Marriott site was

discontinued in December 2006 as the result of too much sampler tampering. A site in Gongna Cove site was subsequently added in its stead.

Ypao Beach

This site is an intertidal seep field, approximately 100 m long, which is exposed at low tide and emerges from the outcropping beach rock. It extends southeast of Ypao Point between the Hilton hotel property to the southernmost Ypao Beach pavilion. This seep field is estimated to produce about 7.6 x 10^3 m^3/day (Jocson, 1998).

Initially at this location, a hole was dug in the sand and coral rubble in the path of greatest flow, and the sampler was buried in this hole, with the GAC bugs on the underside of the concrete block. However, this section of beach is highly trafficked, and children especially like to rearrange the rubble in the seep field. As a result, samplers could not be located during several field events throughout the beginning of the sampling year. In these instances, a new sampler had to be constructed and placed.

Eventually, in November 2006, the placement of samplers at this location was modified, and GAC bugs were thereafter attached to a massive concrete block submerged in the path of fresh water flow, approximately 1 m north of the original sampling site. This solution had its pros and cons – on one hand, the block was too heavy to be moved so the sampling location was spatially constant; on the other hand, the seep expressed itself in different portions of the shoreline so the concentration of fresh water passing by this sampler changed daily.

Due to the coral rubble, limestone bedrock and the nature of flow in this vicinity, it is unlikely that the lost samplers simply settled deeper in the substrate. It is more likely that they were moved from their original locations and may still be lying somewhere nearby. When the very first sampler was placed for this project, an old sampler of Dave Moran's, several years old, was found -- with the bugs still attached. This bug was analyzed with the rest, but no dyes were detected.

Hyatt Spring

North of Ypao Beach, in front of the Hyatt Hotel, there are several small subtidal and intertidal springs, the most prominent of which was chosen as the sampling site at this location. Moran and Jenson (2004) reported that when a PVC pipe was pushed down into one of these springs, roughly 6 cm of head was revealed. Fresh water discharges from fissures and voids in the reef flat pavement just beneath the sand, and boils from the larger springs can be seen on the water's surface when the breeze subsides. At this location, a sampler was buried in one of the larger sandy boils. These small springs push their way through several centimeters of sand from the bedrock below, and the samplers tended to settle quite a bit, making their location and retrieval difficult at times. The original sampler at this site was lost, and a new one was constructed and placed, with rebar attached for metal detection if necessary.

Marriott Spring

Another smaller, intertidal seep field, approximately 3 m across and exposed at low tide on the beach face, is located north of the Hyatt in front of the Marriott Hotel. Like the other seep fields along Tumon Bay, this site is estimated to produce in excess of 10^3 m^3 of fresh water per day (Jocson, 1998).

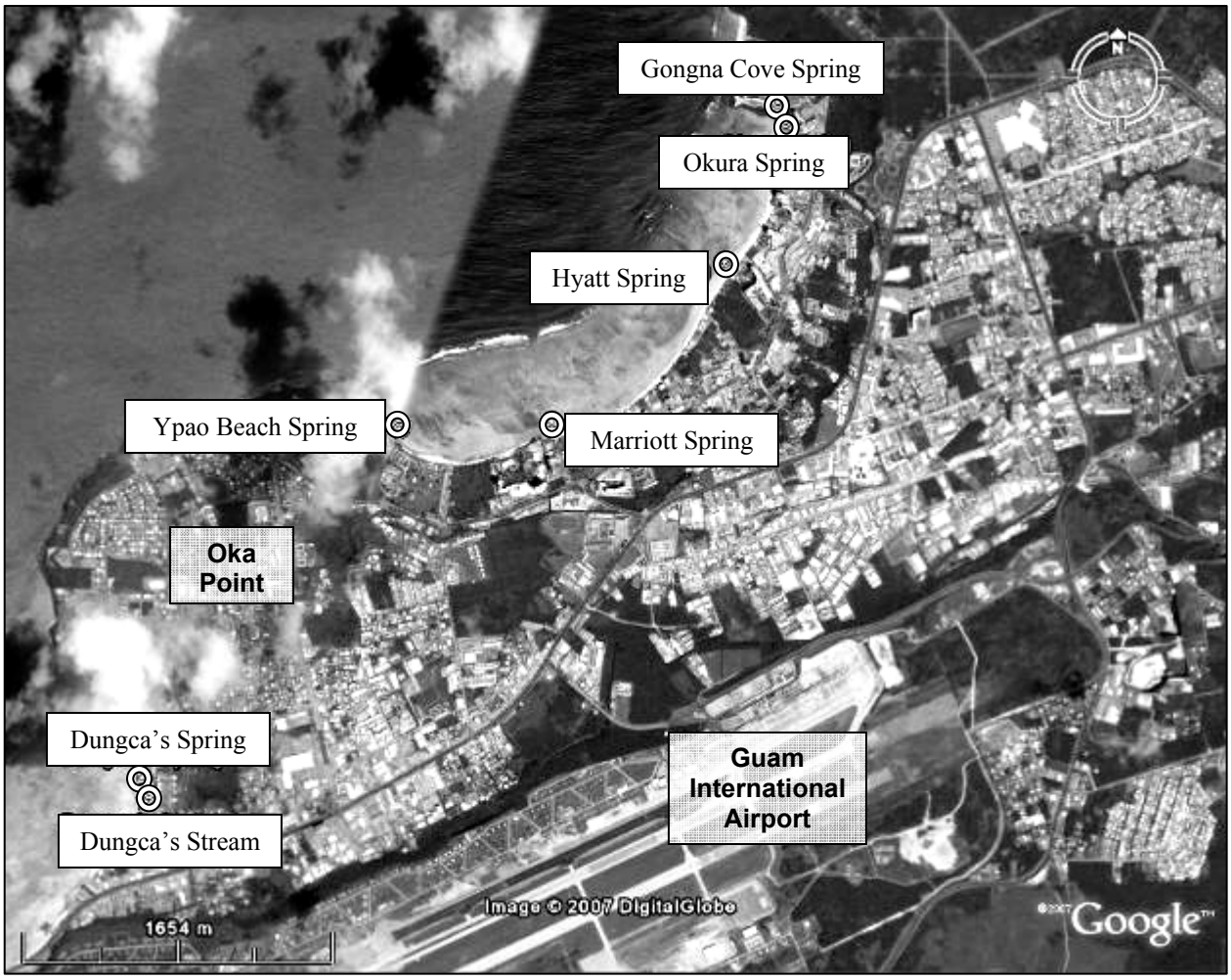

Figure 7. Satellite image of sampling locations in Agana and Tumon Bays. Map source: Google Earth, 2007.

A hole was dug in the sand and coral rubble in the path of greatest flow, and the sampler was buried in this hole, with the GAC bugs on the underside of the concrete block. This section of beach is also highly trafficked. Recreational watercrafts are tethered within meters of the section of shoreline containing the seep field. Several times throughout the year during field events to retrieve and replace bugs, the sampler was either found lying on the beach upturned, or could not be located at all. In November, a new sampler, fitted with a piece of rebar, was constructed and placed; surprisingly, this one too was also lost. Due to the coral rubble, limestone bedrock and the nature of flow in this vicinity, it is unlikely that the samplers settled deeper in the substrate. It is more likely that the missing samplers were either moved or removed by tourists or hotel staff. Since this location experienced a persistent problem with sampler tampering, it was discontinued from the sampling program in December 2006.

Okura Spring and Gongna Cove

Two sampling sites were located in front of the Okura Hotel: a subtidal spring in front of the hotel, and an intertidal spring located in Gongna Cove below the cliff on which the Nikko Hotel sits. The latter site was added in December 2006 upon discontinuation of the Marriott site.

The samplers were buried in the sediment overlying the springs. These small springs push their way through several centimeters of sand from the bedrock below, causing the samplers to settle a bit and making their location and retrieval difficult at times, especially at the site in front of the Okura. The samplers at the site in front of the Okura were lost in August and September 2006, so a new one was constructed and placed, with rebar to aid in relocation.

Agana Bay

Two sampling locations were chosen in Agana Bay: Dungca's Stream and Dungca's Spring.

Dungca's Stream

Dungca's Stream is a shallow channel, about 3.5 m wide along the reach where it was sampled. The channel mouth is located just south of Jimmy Dee's Beach Bar. The head of this stream is covered by development as it regresses inland and underground. Its source is unknown. Although this stream occasionally experiences low flow conditions, it never dried up during the period of study. Bugs were placed in a two-inch diameter, grey PVC tube for protection from direct sunlight and floating debris, with evenly-spaced, 1-in. holes drilled into it for optimal water flow. The tube was strung across a shaded section of the channel with nylon rope so that it would be submerged in the stream flow at all times.

Dungca's Spring

Dungca's Spring is a relatively large, subtidal spring located in Agana Bay directly in front of Jimmy Dee's, about 400 m north of the mouth of Dungca's Stream. Jocson (1998) estimated spring discharge to be several 10^3 m^3 per day. A concrete block was already submerged in the deepest part of the spring, with a rope and buoy attached to it. This proved to be an ideal means of placing GAC bugs, which were attached to a knot in the rope with a zip tie. Bugs at this site were lost on only two occasions throughout the course of the sampling year, presumably removed by tourists, local residents, or watercraft rental staff.

East Coast

Two spring sampling locations were chosen on the east coast (Figure 8), in Pago Bay and Togcha Bay, which are not ordinarily sampled during dye trace studies. These sites were chosen due to the potential influence of specific environmental conditions (*e.g.*, landfill leachate and wastewater effluent, respectively).

Pago Bay

A subtidal spring is located in Pago Bay, south of the Pago River channel on the eastern coast of Guam. This site was chosen because it is located down-gradient from the Ordot Landfill, and the bay likely receives leachate effluent which could contaminate the waters surrounding the spring. A large boulder rests atop the spring vent. A concrete sampler was placed over the point where cool water discharge could be felt most readily. It was not necessary to use rebar at this site, as the sampler was always easy to find. It was discovered overturned on a few occasions, probably by fishermen searching for invertebrates in the crevices around the boulder.

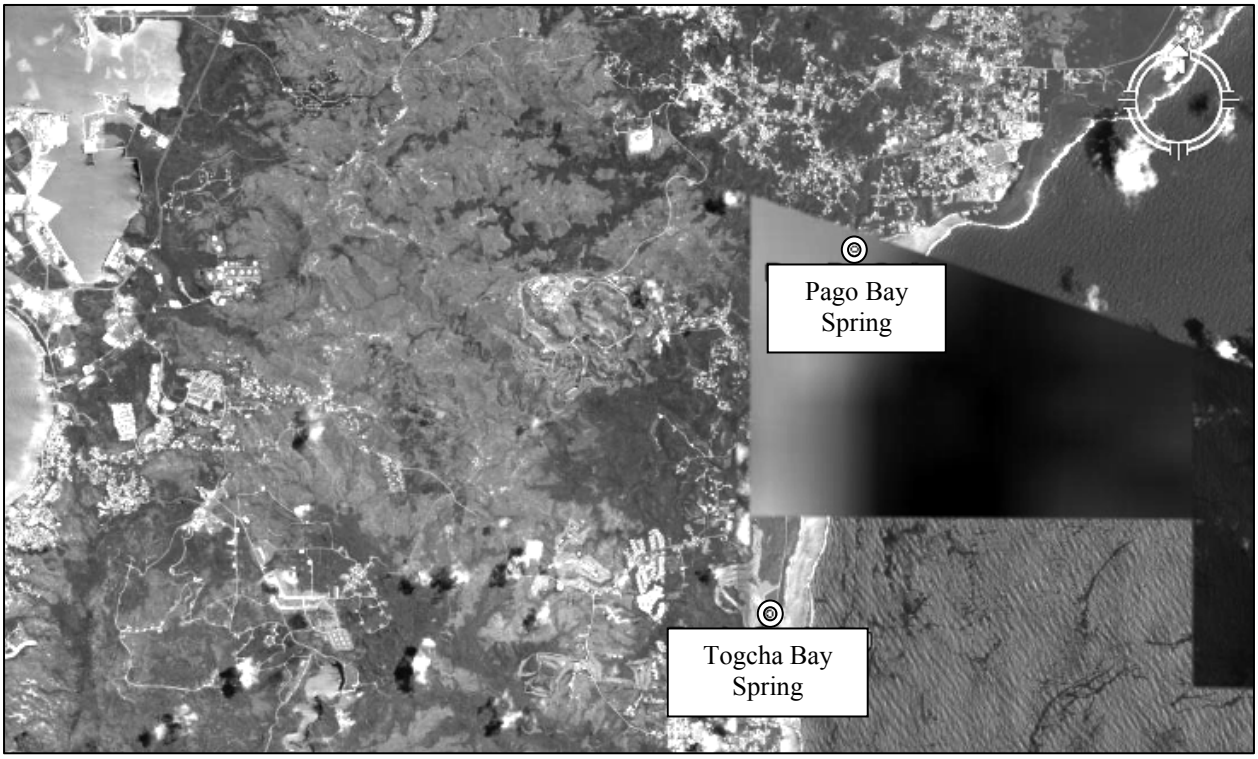

Figure 8. Satellite image of sampling locations on east coast. Map source: Google Earth, 2007.

Togcha Bay

Another subtidal spring on the eastern coast of Guam is located just north of the mouth of the Togcha River. This site was chosen because the Togcha River receives treated wastewater effluent from a wastewater treatment plant about 2.7 km upstream. As such, the lagoon surrounding the river mouth receives effluent enriched in fluorescent dyes, especially those in the range of optical brighteners (Hoffman, unpublished).

During low tide, fresh water can be observed (as surface slicks from a distance and as cloudiness close up) discharging from holes in the bedrock substrate. The sampler was placed in a fissure in the exposed limestone bedrock. For the first few months of the sampling year, it was not necessary to use rebar at this site, as the sampler was always easy to find. It was discovered over-turned on the shore on one occasion, probably by fishermen searching for invertebrates in the crevices around the bedrock. Later, during a sampling round in November, it was discovered that the hole in which the sampler had been placed had widened -- perhaps due either to natural collapse or to the disturbance by fishermen. The sampler could not be located, and so a new sampler was constructed and placed in the hole. The new sampler was fitted with a length of rebar wider than the vent to suspend the sampler across the aperture and prevent it from becoming lost down the hole.

Control Sites

Asma Fenas River

Control samples were obtained from the Asma Fenas River (Figure 9), a surface stream in the volcanic physiographic province of southern Guam. The river receives groundwater discharge from contact springs along the edges of a perched limestone aquifer on the flanks of Mount LamLam. Samples were collected from approximately 100 to 200 meters below the headwaters of the stream. Due to the lack of anthropogenic influences from above, water discharging from this stream is assumed to reflect unspoiled groundwater sharing the same freshwater input source (rainfall) as water discharging in the NGLA, but without the influence of surrounding seawater.

Bugs were placed in a two-inch diameter, grey PVC tube for protection from direct sunlight and floating debris, with evenly-spaced, one-inch holes drilled into it for optimal water flow. The tube was submerged in a pool at the base of a small waterfall, and secured to a tree trunk with nylon rope of sufficient length so that it would be submerged at all times but not washed away during high flow events.

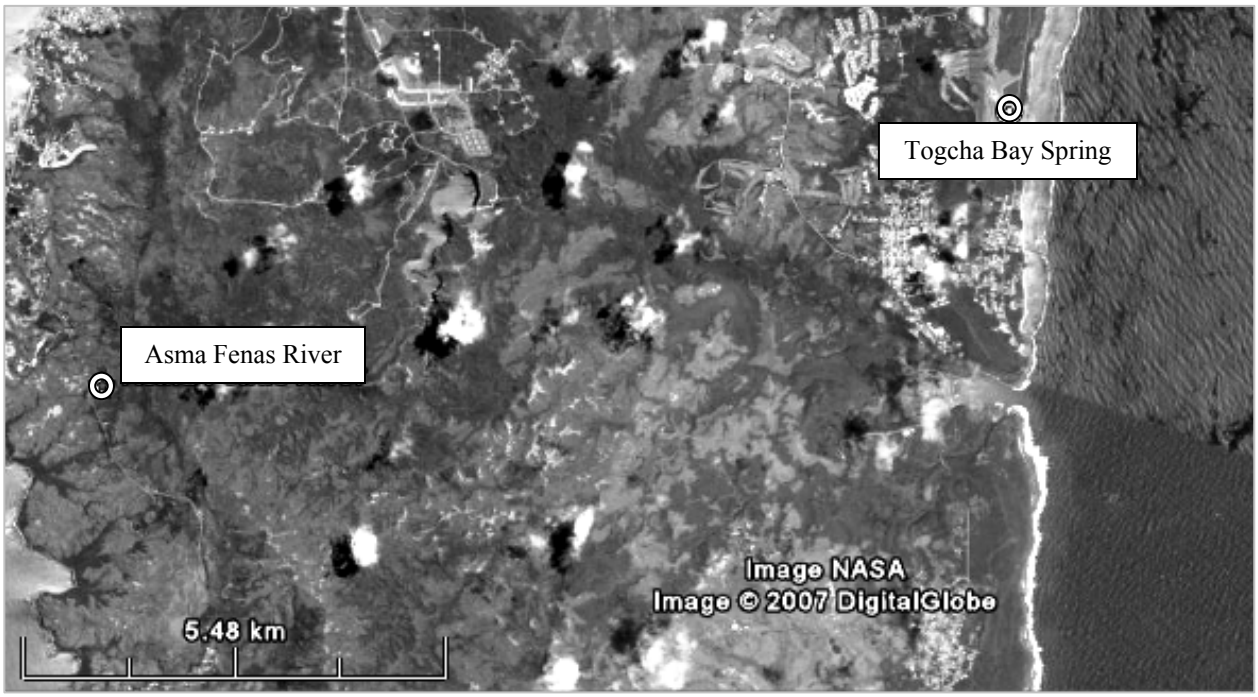

Figure 9. Asma Fenas River sampling site location. Map source: Google Earth, 2007.

Ambient Seawater

Two sets of GAC samples of ambient seawater were collected from four locations around central Guam (Figure 10, Table 1) to account for background levels in the surrounding nearshore environment. These locations were chosen due to their proximity to the majority of the freshwater discharge locations. In addition, a total of 31 grab samples were randomly collected from the seawater immediately surrounding each of the 13 monitoring locations.

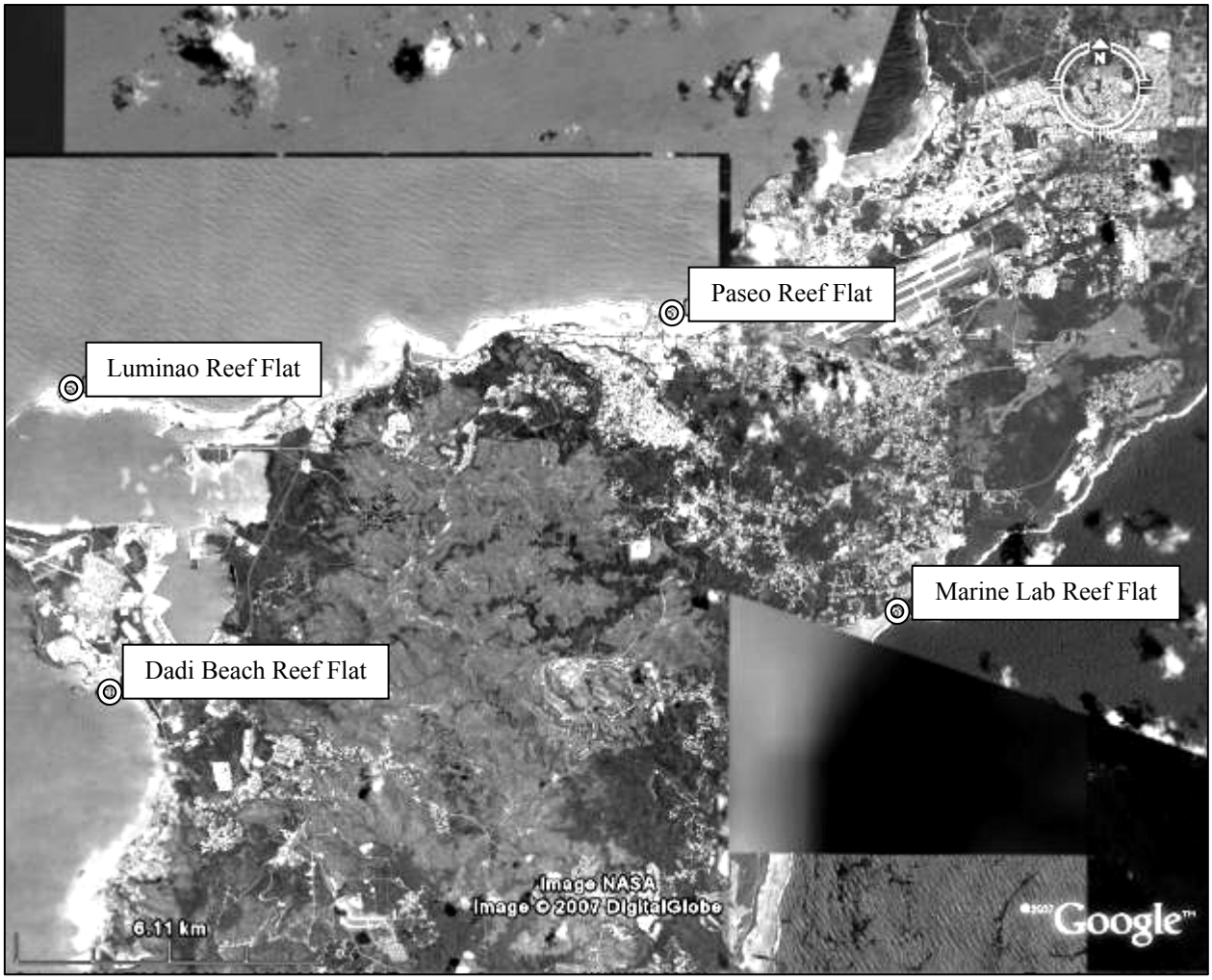

Figure 10. Satellite image of ambient seawater sampling locations. Map source: Google Earth, 2007.

Saipan

A personal travel opportunity, related to part-time employment independent of this project, presented the opportunity to collect samples from two coastal springs on Saipan (Figure 11, Table 1) for comparison. Saipan's hydrogeology is similar to the NGLA in many respects. Likewise, literature research has revealed no evidence of dye trace studies having been conducted on the island. As such, it was presumed that groundwater discharging into the Saipan lagoon may be proxy for background levels on Guam in the absence of previous dye trace studies. Only a few samples were able to be collected in Saipan, at two locations, and samplers were sometimes left *in situ* for several weeks to months. Evidence suggests that the longer a bug is left in the environment, the lower its ability to adsorb fluorescent materials (Aley, 1999; Smart and Karunaratne, 2002). As such, the data obtained from these locations have been used strictly for qualitative comparison.

Freshwater inputs to Saipan's coastal waters include direct rainfall, seaward-flowing groundwater, and overland flow. Rainfall in the island's interior contributes to both groundwater discharge and

overland flow. Overland flow upgradient of these springs could contribute a significant input of freshwater to coastal waters during periods of intense rainfall. Tidal fluctuations are also reflected in groundwater levels, and at any given time groundwater levels are higher than sea level (Perrault, 2007).

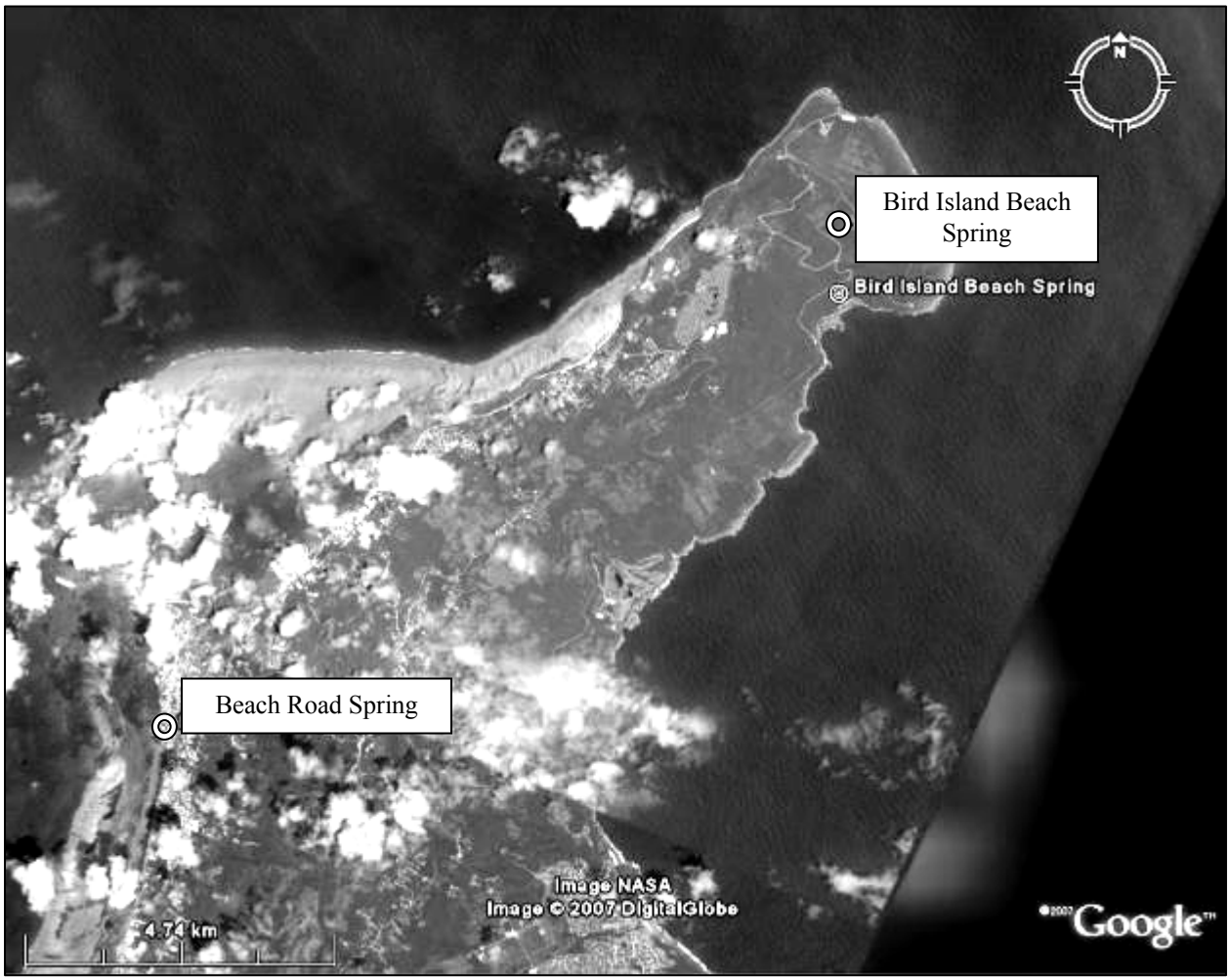

Figure 11. Satellite image of Saipan sampling locations. Map source: Google Earth, 2007.

Although the population has historically been concentrated in the south and southwest, development has shifted since World War II toward the central part of the island. The village of Garapan has become a center of commerce for the island's garment industry. The garment industry utilizes massive quantities of various dyes, some of which are closely related to those used for dye tracing studies. Invariably, these dyes get incorporated into wastewater effluent and stormwater runoff, which could then get injected into the aquifer and discharge in coastal springs. Numerous private production and injection wells have been installed on hotel properties adjacent to the Beach Road spring site, and are essentially unmonitored by regulatory agencies with regard to the water quality and rates of withdrawal or injection (Perrault, 2007).

Bird Island Beach Spring

An intertidal spring is located on Bird Island Beach against a rocky outcrop at the southern end of the beach. A hole was dug in the sand and coral rubble in the path of greatest flow, and the sampler was buried in this hole, with the GAC bugs attached to the underside of the concrete block. This beach is relatively remote, accessible only by a trail which descends along the cliff face through the jungle, so it was assumed that the sampler was never tampered with. It did, however, get buried beneath the sediment between field visits, making it difficult to locate and retrieve, but also keeping it protected from sunlight and continually immersed in water.

Beach Road Spring

This subtidal spring is located offshore from Beach Road in Garapan, on the west-central coast of Saipan south of a concrete dock called "Fishing Base". This location was sampled four times between April and July 2006. GAC bugs were attached using zip-ties directly to a "keyhole" in a fissure in the bedrock at the spring's vent, which is elliptical and approximately 1 m wide. The size of this spring vent is between that of Dungca's Spring in Agana Bay and the Okura spring in Tumon Bay. Therefore, the volume of fresh water discharging from this site is estimated to be similar, on the order of 10^3 m^3/day.

Laboratory Analysis

A multitude of technical reports, scientific journal articles and other documents have been published over the last 35 years (Smart, 1972; Smart and Smith, 1976; Quinlan, 1987; Smart, 1988; Alexander and Quinlan, 1992; Aley, 1999; Smart and Karunaratne, 2002; Smart and Simpson, 2002; Aley, 2003; Moran and Jenson, 2004; Otz *et al.*, 2004; Hagedorn *et al.*, 2005) describing effective and reliable procedures for designing tracer studies, handling and analyzing samples, and interpreting the data derived from such studies. Although this study was not an actual dye trace, samples were collected and analyzed following guidelines consistent with these documents.

Field samples were analyzed using the Cary Eclipse fluorescence spectrofluorometer by both a synchronous scanning protocol (SSP) and multi-wavelength analysis (MWA). Specific parameters used for each method are provided in Appendix C. SSP provided graphic data which were used to determine whether results obtained during MWA were just background interference (noise) or could be attributed to a particular dye. MWA provided fluorescence intensity data at specified wavelengths (corresponding to each of the four dyes of interest). Results obtained from analysis of eluted charcoal samples are analyzed and discussed in detail in the next chapter.

Synchronous Scanning Protocol

Each field sample was scanned using SSP to get a graphic representation of the sample and its constituents. Dye standards run using SSP revealed that peaks on the graphs represented excitation wavelengths, not emission wavelengths. Graphs obtained from samples were studied to find well-defined peaks which corresponded to the known excitation wavelengths of each dye.

Multi-wavelength Analysis

Raw data obtained from analysis of all field samples using the Cary Eclipse fluorescence spectrophotometer have been tabulated and are presented in Appendix D. Raw data acquired from this instrument were expressed as arbitrary units (a.u.) of relative fluorescence intensity on a scale of 0 to 1000 for all four dyes of interest. Prior to analysis, concentration curves (Figures 12 and 13) were established for each dye in both eluent and water. Concentrations (C) were positively, linearly correlated to intensity (I). Formulae used to express raw data in concentrations of ppb in solution (micrograms per litre, or µg/L) are shown in Table 2.

Table 2. Concentration curve formulae derived from analysis of standard solutions of fluorescent materials of interest in both eluent and water

Fluorescent Material	Concentration Range (ppb)	Concentration in Eluent	Concentration in Water
Optical Brightener	0 to 0.100	$\frac{I - 34.59}{6.26}$	$\frac{I - 34.59}{6.26}$
Sodium Fluorescein	0 to 100	$\frac{I + 18.46}{150.85}$	$\frac{I - 1.71}{0.99}$
Eosine Y	0 to 500	$\frac{I - 57.53}{0.52}$	$\frac{I - 51.71}{4.28}$
Rhodamine WT	0 to 250	$\frac{I - 0.12}{81.56}$	$\frac{I - 26.39}{10.20}$

The data were then normalized with respect to the mass of charcoal eluted for each sample and converted to nanograms of dye per gram (ng/g, or ppb) of dry charcoal. This was accomplished by multiplying the aqueous concentration by the mass of the GAC and dividing by the volume of eluent used. For example, an intensity value (*I*) for rhodamine of 405.26 for a dried sample weighing 11 grams and eluted with 30 mL of eluent would yield a calculated concentration of 13.5 ng/g:

Materials and Equipment Used

A Cary Eclipse spectrofluorophotometer, standard laboratory glassware and disposable polystyrene cuvettes were used to prepare and analyze eluted samples fluorometrically. A caustic solution of aqueous ammonia, isopropanol, reverse-osmosis (RO) water and potassium hydroxide was used to elute adsorbed dyes from the GAC and enhance fluorescent intensity. To prevent etching, as well as cross-contamination, all glassware was washed after each use using a solution of Alconox® and rinsed with isopropanol and RO water.

Instrument Calibration and Quality Control

The spectrofluorometer was calibrated using standard solutions of sodium fluorescein, eosine Y, rhodamine WT, and optical brightener (in solution in household laundry detergent). Standards, both eluent-based and water-based, were formulated in the laboratory using materials as sold by the manufacturers (crystalline or powdered for fluorescein and eosine, aqueous solutions of rhodamine and optical brightener).

Prior to sample analysis, the instrument's detection limits for each fluorescent material of interest were determined by analyzing serial dilutions of each of the standard solutions, as well as laboratory samples spiked with known concentrations of each dye, to determine the lowest concentration which yielded a detection response three times the signal-to-noise ratio of the machine. Although the instrument's detection limits proved much lower, conservative method detection limits (MDLs) were used to account for the effects of other fluorescent materials routinely encountered in field samples and other environmental factors. MDLs used for fluorescein, eosine and rhodamine WT, respectively, are as follows

(after Aley, 1999): in water – 0.0005, 0.0050 and 0.0070 ppb; and in elutant – 0.010, 0.040 and 0.155 ppb.

The instrument was "zeroed" using reverse-osmosis (RO) water and eluent in polystyrene cuvettes prior to analysis of each batch of samples. Both liquids were analyzed on multiple occasions and exhibited negligible fluorescence. The instrument was set up according to the manufacturer's specifications and recommendations. Methods, outlined in Appendix C, were programmed into the machine and used consistently throughout the entire study. Four calibration standards of each dye were prepared fresh twice during the period of laboratory analysis – once in August 2006 and again in January 2007 – in concentrations ranging from 0.2 ppb to 70 ppb. Standards were analyzed before and after each batch of samples analyzed.

Sample Preparation

Preparation and Analysis of GAC Samples

GAC bugs were rinsed vigorously upon collection in the waters of their respective sampling environments. They were then wrapped in aluminum foil to protect the contents from photodegradation and cross-contamination, labeled using a black permanent marker, and chilled during transport to the laboratory. Samples which were not analyzed immediately were refrigerated until analysis to inhibit biological activity and prevent photodegradation.

In the lab, samples were prepared for analysis according to the following procedure (after Mull *et al*., 1988 and Aley, 1999): (1) mesh bags were rinsed vigorously with RO water using a small pump sprayer to remove charcoal fines, sediment and biofilm; (2) bugs were then rewrapped loosely in their original, labeled foil wrappers and set in a drying oven set at 50ºC for approximately 24 hours; (3) once dry, bugs were cut open one at a time, the dried charcoal from each was removed and weighed using a digital scale (accurate to ±0.001 gram), and the charcoal was then deposited into 40-mL, amber glass vials; (4) 15 mL of eluent was added to each vial, the vial capped and shaken gently to release trapped air pockets and saturate the charcoal grains; (5) sample vials were allowed to sit for one hour, with occasional gentle agitation to promote saturation of charcoal; (6) aliquots of 3.5 mL were drawn one at a time from each vial, transferred into a polystyrene cuvette and placed in the spectrofluorometer for analysis.

Elution of fluorescent compounds from the GAC was achieved using a supersaturated, alkaline solution consisting of potassium hydroxide (KOH) pellets dissolved in a solution of 5% aqueous ammonia, 66.5% 2-propanol (isopropyl alcohol), and 28.5% RO water (after Quinlan, 1987). Results obtained from analysis of eluted charcoal samples are discussed in the next chapter. After analysis, leftover elutant from each pair of bugs were combined into a single vial and stored in a refrigerator in case any were needed for re-analysis.

Preparation and Analysis of Water Samples

Grab samples were randomly collected from the waters of each sampling site using 40-mL, amber, borosilicate glass vials capped with plastic, Teflon®-lined lids. A trip blank was created for each sampling event to account for any contamination not associated with the sampling sites themselves. Each sample was labeled, capped and chilled immediately after collection to inhibit biological activity and prevent photodegradation.

Optimal wavelengths of each dye in both water and eluent were obtained prior to analysis. Wavelengths obtained for each dye in water proved to be slightly different than those for eluent (Table 3). As a result, the same SSP parameters utilized for GAC elutant samples were also used for grab water samples, but a higher detector setting and slightly different excitation and emission wavelengths were employed in MWA (see Appendix C). Aliquots of 3.5 mL were drawn one at a time from each sample vial, transferred into a polystyrene cuvette and placed in the spectrofluorometer for analysis.

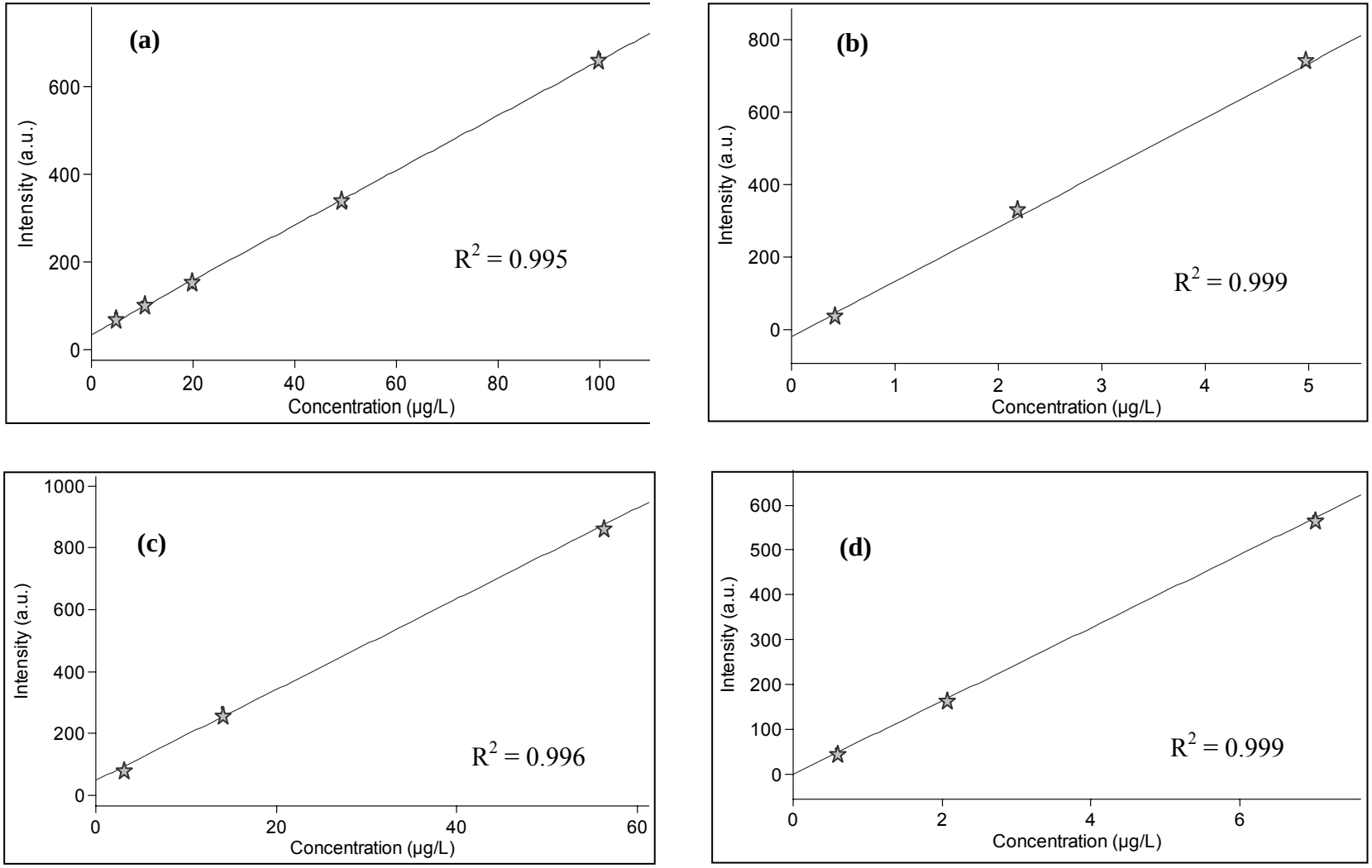

Figure 12. Concentration calibration curves of dye standards in eluent: (a) optical brightener; (b) sodium fluorescein; (c) eosine Y; and (d) rhodamine WT.

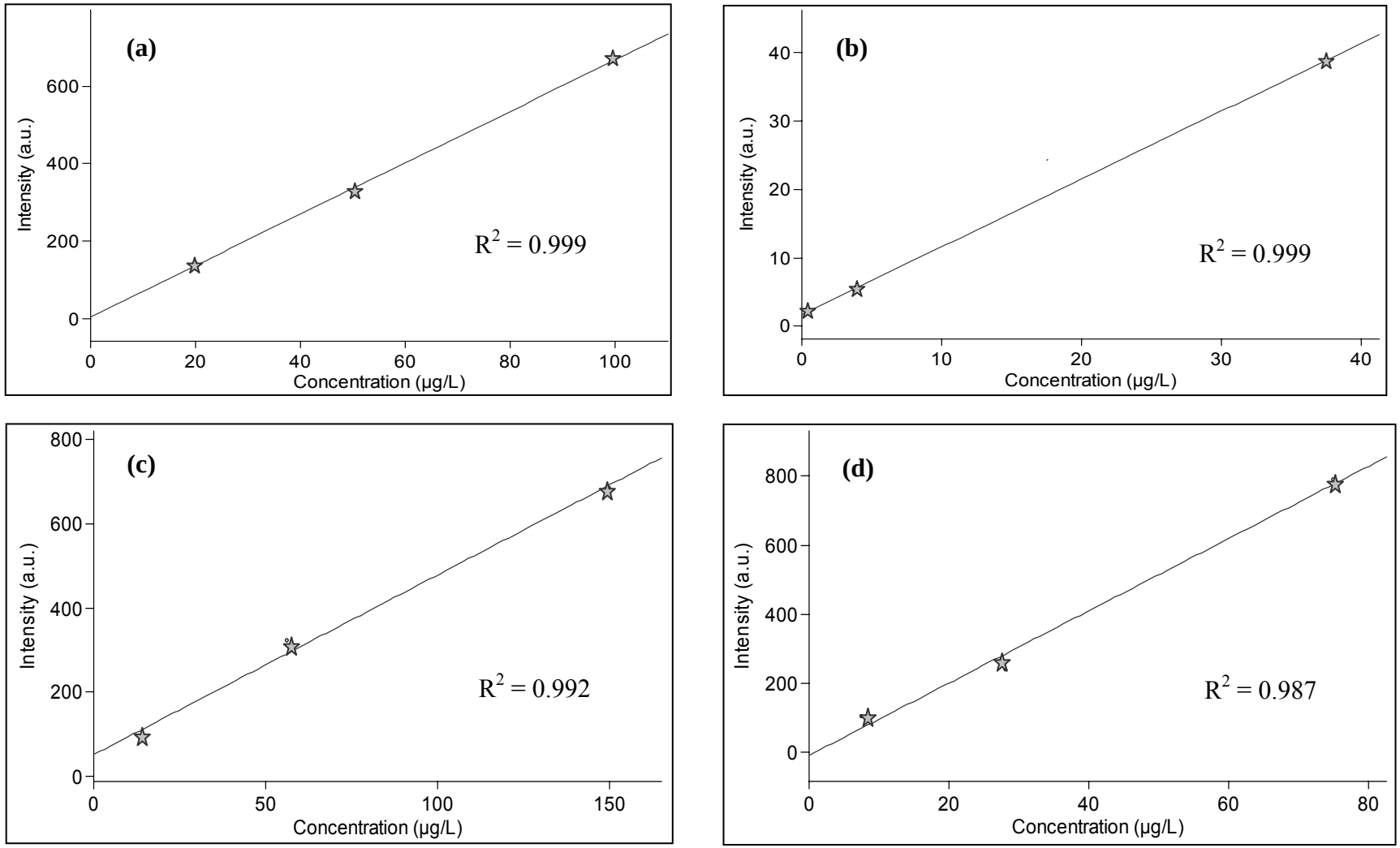

Figure 13. Concentration calibration curves for dyes in water: (a) optical brightener in detergent; (b) sodium fluorescein; (c) eosine Y; and (d) rhodamine WT.

Table 3. Optimal excitation and emission wavelengths for fluorescent materials of interest.

			OBSERVED IN					
	LITERATURE[1]		2-PROPANOL		ELUENT		WATER	
Compound Name	EX (nm)	EM (nm)	EX (nm)	EM (nm)	EX (nm)	EM (nm)	EX (nm)	EM (nm)
Sodium Fluorescein	491	512	490	515	500	520	495	515
Eosine Y	516	538	538	551	525	545	515	535
Rhodamine WT	554	580	548	569	550	570	555	575
Phorwite BBH Pure[2]	349	430	optical brightener in detergent solution[3]					
Tinopal ABP Liquid	350	435	360	432	350	430	345	425

Notes:

[1] *from Käss 1998 (values in aqueous solution)*

[2] *a.k.a. Fluorescent Brightener #28, Calcofluor White ST, Tinopal LPW, and Tinopal 4BM*

[3] *used diluted commercial laundry detergent to obtain observed values*

After analysis, the pH for each grab sample was measured using a waterproof, pen-type, PH-03(II) meter and recorded. The meter was calibrated using buffer reference standards of pH 7.00 and 10.00 (±0.01 @ 25°C) obtained from Sigma Chemical Co. in St. Louis, Missouri. Leftover grab samples were discarded. Results obtained from analysis of grab samples are discussed in the next chapter.

RESULTS AND DISCUSSION

Field Samples

Dry weight concentrations were calculated for each dye over time. The data were condensed according to region, and descriptive statistics for each region were calculated. Island-wide means are presented in Figure 14. The data was normally distributed. A two-way analysis of variance (ANOVA) was performed to determine whether the data varied significantly with respect to location or time of year. Spatial and temporal variability were analyzed, and other sources of variability (*e.g.*, sampling substrate and precipitation) were investigated.

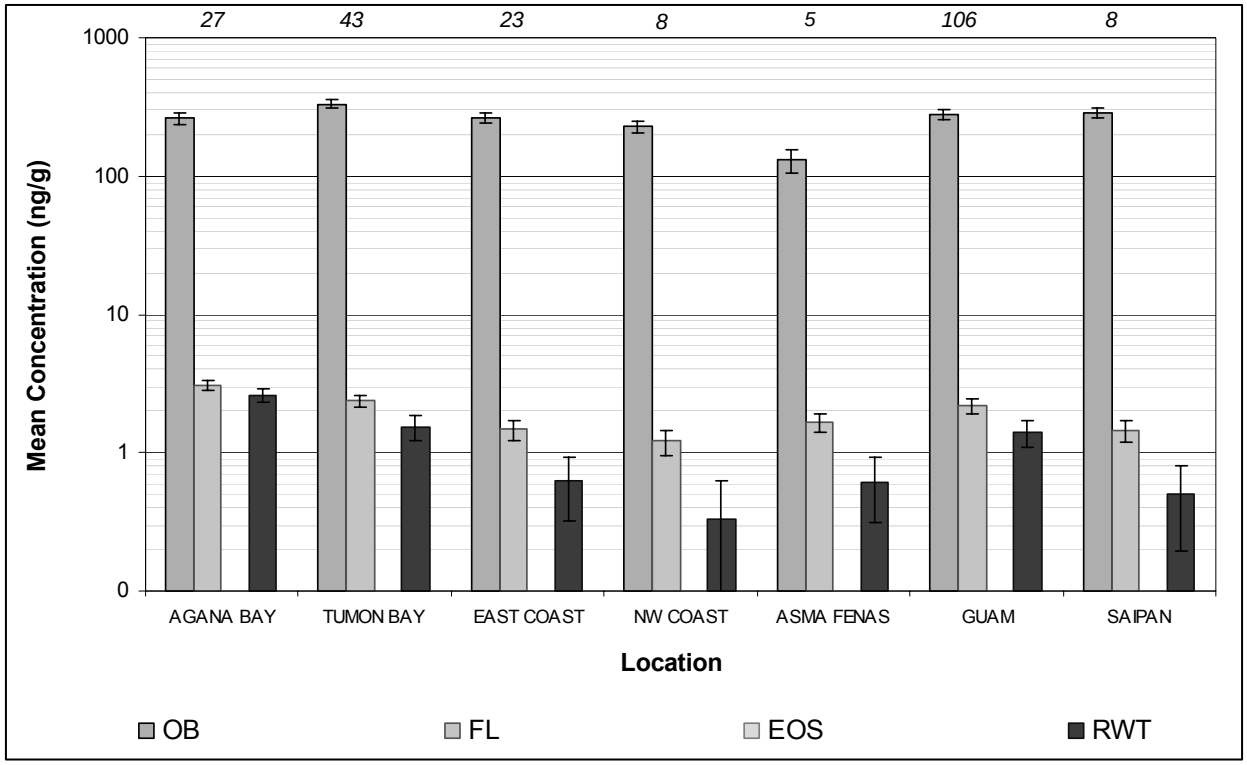

Figure 14. Mean equivalent dry weight concentrations (ppb) from island sampling regions during period of study. OB = optical brighteners, FL = fluorescein, EOS = eosine, and RWT = rhodamine. FL and RWT are geometric means. Error bars represent standard error. Italicized numbers along top represent # of samples for that region. Eosine was rarely detected.

Data Analysis

Despite careful planning, several sampling sites lack data for given sampling rounds. This was due either to unsynchronized sampling schedules, or unforeseeable events including missing or damaged samplers and inclement weather conditions. The resulting unequal sample sizes could be handled one of three ways (Quinn and Keough, 2002). In the first, no action is taken; in ANOVA, this means that variances are heterogeneous and group means are estimated with different levels of precision, making interpretation difficult. In the second, all observations are deleted for a sample containing missing

observations, although using this approach squanders data and decreases the power of the statistical test. The third approach substitutes replacement values (*i.e.*, a mean or randomly generated number) for the missing observations, although this method underestimates variance and standard error, returning fallaciously precise results.

In preliminary analyses, the three approaches yielded different results using a two-way ANOVA without replication. Due to the shortcomings of the other two, the first approach was chosen for subsequent analysis. A two-way ANOVA (without replication) was performed where factor A was site location and factor B was time of year. The null hypotheses were that background concentration was unaffected by either location or time of year. Table 4 summarizes the results of the ANOVA, which reveal that variability of background concentrations is significant both spatially and temporally.

Table 4. Summary of ANOVA results. All $p << 0.001$. FMOI = fluorescent material of interest. OB = optical brighteners, FL = fluorescein, and RWT = rhodamine. Numbers in parentheses indicate degrees of freedom.

FMOI (*df*)	F	P
OB	7.52	$2.81e^{-6}$
Location (6,72)	3.77	$1.92e^{-4}$
Time (12,72)		
FL	11.67	$2.79e^{-10}$
Location (7,84)	4.73	$7.56e^{-6}$
Time (12,84)		
RWT	9.21	$2.08e^{-8}$
Location (7,84)	3.21	$8.07e^{-4}$
Time (12,84)		

Physical Properties of Elutants

The contents of all bugs were weighed after drying and prior to elution. The mass of charcoal plus adsorbed materials in each bug ranged from 3.97 g to 7.93 g, averaging 5.81±0.73 g. Each elutant was also observed for color and clarity, and graded using the following system:

- Color: colorless; almost colorless; very pale yellow; pale yellow; light, bright yellow
- Clarity: clear; contains colloids; contains fine GAC sediment; cloudy

Only eluted samples from the northwest coast and the Asma Fenas River were colorless during the period of study. All elutants from Tumon and Agana Bay samples were colored to some extent, suggesting the influence of stormwater runoff from these highly developed areas. Many samples contained GAC fines or colloids; this was not related to sampling location or time of year, but rather to the batch of samples processed for analysis.

Multi-wavelength Analysis

A summary of the pooled data obtained from field sampling is presented in Tables 5 and 6, according to island region and season, respectively. These results represent time-integrated concentrations based on varying deployment intervals. Ranges and arithmetic averages for each region are presented in the following sections. Eosine was not detected in the majority of samples above detection limits. Eosine data appeared to fit a Poisson distribution.

Table 5. **Summary of dry-weight concentrations of fluorescent materials of interest in samples, grouped by island region.**

Region	Statistic	Calculated Concentration (ng/g, ppb)			
		Optical Brightener	Sodium Fluorescein	Eosine Y	Rhodamine WT/B
AGANA BAY	mean	261	3.08	<0.040	2.60
	max	414	7.01	574	14.1
	min	109	1.19	<0.040	0.452
	std. dev. (σ)	67.7	1.30	256.42	3.01
	var.(σ^2)	4579	1.68	65753	9.04
TUMON BAY	mean	336	2.39	<0.040	1.56
	max	532	4.37	22.9	3.15
	min	192	0.891	<0.040	0.268
	std. dev. (σ)	75.2	0.813	141	0.761
	var.(σ^2)	5650	0.662	<0.040	0.580
NW COAST	mean	227	1.21	<0.040	0.328
	max	669	1.88	<0.040	0.666
	min	<0.100	0.626	<0.040	0.041
	std. dev. (σ)	225	0.475	120	0.216
	var.(σ^2)	50848	0.226	14295	0.047
EAST COAST	mean	265	1.48	<0.040	0.63
	max	377	2.30	<0.040	1.62
	min	116	0.909	<0.040	0.275
	std. dev. (σ)	58.6	0.333	85.76	0.285
	var.(σ^2)	3438	0.111	7355	0.081
ASMA FENAS	mean	287	1.44	<0.040	0.504
	max	637	4.23	<0.040	1.15
	min	79.2	0.849	<0.040	0.132
	std. dev. (σ)	42.3	0.702	167	0.451
	var.(σ^2)	1791	0.492	27852	0.203
GUAM	mean	271	2.22	<0.040	1.43
	max	669	7.01	574	14.1
	min	<0.100	0.626	<0.040	0.041
	std. dev. (σ)	111	1.06	280	1.74
	var.(σ^2)	12213	1.13	78482	3.01
SEA-WATER	mean	110	2.00	<0.040	0.332
	max	135	2.12	<0.040	0.498
	min	70.3	1.87	<0.040	0.198
	std. dev. (σ)	20.7	0.074	24.0	0.079
	var.(σ^2)	430	0.005	574	0.006
SAIPAN	mean	130	1.67	<0.040	0.616
	max	196	2.71	<0.040	1.30
	min	56.6	0.896	<0.040	0.259
	std. dev. (σ)	136	0.814	721	0.263
	var.(σ^2)	18493	0.663	520120	0.069

Table 6. Summary of dry-weight concentrations of fluorescent materials of interest in samples, grouped by wet (June through November) and dry season (December through May).

		Calculated Concentration (ng/g, ppb)			
	Statistic	Optical Brightener	Sodium Fluorescein	Eosine Y	Rhodamine WT/B
WET	mean	285	2.19	<0.040	1.16
	max	637	6.51	46.0	4.75
	min	56.6	0.849	<0.040	0.132
	std. dev. (σ)	103	1.09	0.000	0.868
	var.(σ^2)	10650	1.18	125633.17	0.753
DRY	mean	278	2.18	<0.040	1.57
	max	669	7.01	574	14.1
	min	<0.100	0.626	<0.040	0.041
	std. dev. (σ)	112	1.09	0.00	2.11
	var.(σ^2)	12648	1.19	45486	4.44

Wet vs. Dry Season

The few eosine detections that were made occurred during the dry season. Variability (Figure 15) of all fluorescent materials of interest was higher in the dry season, particularly for rhodamine. Standard deviations for each dye during the wet and dry seasons, respectively, were as follows: 103 and 113 for optical brighteners, 1.07 and 1.11 for fluorescein, and 0.836 and 2.22 for rhodamine. Statistical analysis (i.e., two-sample t-tests and two-sample F-tests) revealed that means and variances (Table 6) between dyes did not differ significantly (both tests, $\alpha = 0.05$, $p = 0.00$, 0.00 and 0.00 for all three combinations of optical brighteners, fluorescein and rhodamine). Seasonal variance among each dye did, however, vary significantly (one-way ANOVA, $\alpha = 0.05$, $p = 0.51$, 0.81 and 0.16 for optical brighteners, fluorescein and rhodamine, respectively).

Agana Bay

The two sampling locations in Agana Bay had the highest levels of fluorescence in the range of fluorescein, eosine and rhodamine of all sites throughout the period of study. In addition, these sampling sites showed the greatest variance (σ^2) in the range of fluorescein and rhodamine. For fluorescein, these observations are likely attributable to surface runoff entering Dungca's Stream.

East Coast

Eosine was not detected in any samples from the East Coast sites (Table 4). These sites showed the lowest variance for fluorescent materials in the range of sodium fluorescein and rhodamine WT.

Northwest Coast

Eosine was not detected in any of the samples from the vertical dissolution fractures or flank margin cave located near Double Reef. These three locations had the highest levels of fluorescence, the greatest variance in the range of optical brighteners of all sampling sites. This may be due either to surface runoff or dissolved humic and fulvic compounds. In addition, this site had the lowest levels of fluorescence in the range of optical brighteners, fluorescein and rhodamine.

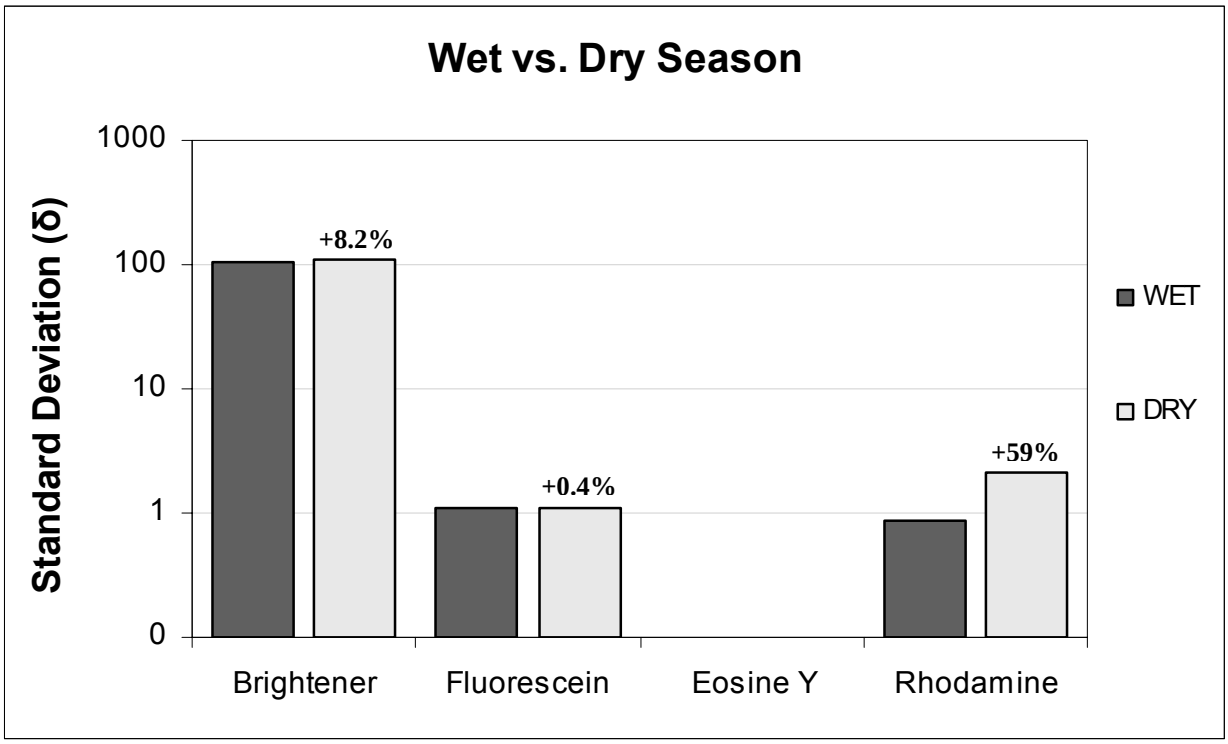

Figure 15. Comparison of standard deviations during wet and dry seasons for fluorescent materials of interest. Numbers above bars indicate increase in variability during dry season.

Asma Fenas River

It was expected that the water in this river would be pristine, and therefore exhibit very low levels of fluorescence similar to the xanthene dyes. This site had the second highest levels of fluorescent materials in the range of optical brighteners during the period of study. This is likely due to dissolved humic and fulvic compounds in the stream. Samples from this river also exhibited the second lowest variances for fluorescent materials in the range of fluorescein and rhodamine. Furthermore, eo-sine was also not detected in any samples from this site.

Variability Between Bugs

Of the 130-plus pairs of bugs analyzed, 83 pairs were randomly chosen to deter-mine the variation in concentration of their respective elutants. Differences between values ranged from 0.3% to 94%, averaging 14±12% (Figure 16).

Synchronous Scanning Protocol

A broad envelope of background noise could overshadow what would otherwise be well-defined peaks (Figure 17). Nearly all samples exhibited such an envelope in the range of optical brighteners (*i.e.*, between 340 nm and 450 nm). Therefore, all MWA concentrations for optical brightener reported for each sample are merely estimates of the background fluorescence at a given wavelength, and not necessarily the concentration of actual dye present in the sample.

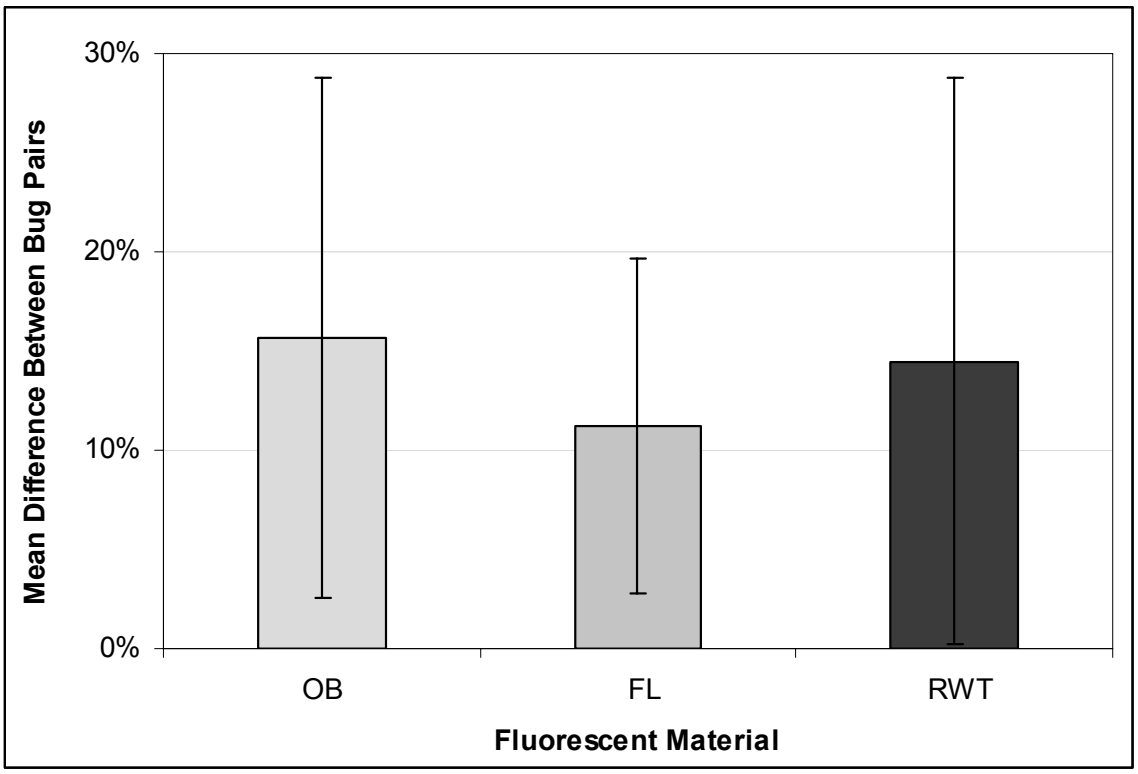

Figure 16. **Variability of results between replicate bugs in the field. OB = optical brightener; FL = fluorescein; and RWT = rhodamine. Error bars represent one standard deviation.**

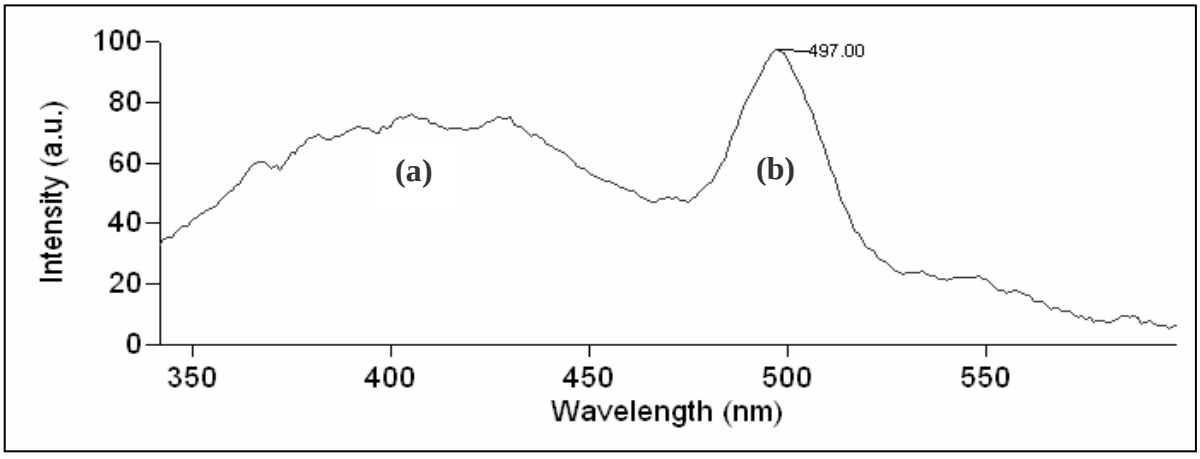

Figure 17. **Example graph showing (a) broad envelope which may be obscuring peaks associated with optical brighteners and (b) well-defined peak associated with sodium fluorescein.**

Positive dye detections were defined by the following two criteria: (1) SSP graph showed a clearly defined peak at the appropriate wavelength; and (2) concentrations calculated from results of MWA analysis exceeded the island-wide mean for a given dye by 3 standard deviations (to encompass 99.7% of all background values). Based on these criteria, fluorescein was detected at Dungca's Stream in June and December 2006 and January 2007. This is likely due to surface runoff. Rhodamine, on the other hand, was detected at Dungca's Spring in March, April, May, September and October 2006. These detections may indicate that the latest injection (2004) is still discharging from the aquifer. No positive detections of optical brightener or eosine were made at any location.

Grab Freshwater Samples and GAC Seawater Samples

Grab samples of spring discharge and GAC samples of seawater were also analyzed using the Cary Eclipse fluorescence spectrofluorometer by both multi-wavelength analysis (MWA) and a synchronous scanning protocol (SSP). After analysis, the pH of each grab sample was measured with a pH meter and recorded. Leftover samples were discarded after analysis. Results are analyzed and discussed in detail in the next chapter. It should be noted that, in contrast to the time-integrated GAC samples, grab samples are instantaneous.

Multi-wavelength Analysis

Fresh Water

A summary of results is presented in Table 7. Means are compared with those of seawater GAC samples in Figure 18. With the exception of optical brighteners, all other dyes are present in higher concentrations in the spring water samples. Fluorescence intensity values for grab water samples were converted to comparable concentrations based on standard calibration curves. Analysis of RO water resulted in calculated background concentrations of 22.5, 0.50, 3.4 and 6.8 ppb for optical brighteners, fluorescein, eosine and rhodamine, respectively. Concentrations of optical brightener ranged from 8.49 to 240 ppb, with a mean of 60.7±44.3 ppb. Fluorescein concentrations varied from 0.50 to 79.8 ppb, averaging 15.1±18.1 ppb. Concentrations of eosine varied from <0.001 to 71.2 ppb, with a mean of 12.1±15.3 ppb. Rhodamine concentrations ranged from 'non-detect' to 39.6 ppb, averaging 8.5±8.4 ppb. Water samples exhibited the greatest variance for all fluorescent materials of interest.

Seawater

A summary of results is presented in Table 8. Means are compared with those of grab samples from submerged springs in Figure 18. With the exception of optical brighteners, all other dyes are present in lower concentrations in the seawater samples. Concentrations of optical brightener ranged from 70.3 to 135 ppb, with a mean of 110±20.7 ppb. Fluorescein concentrations varied from 1.87 to 2.12 ppb, averaging 2.00±0.074 ppb. Concentrations of eosine were 'non-detect'. Rhodamine concentrations ranged from 0.20 to 0.50 ppb, averaging 0.330±0.079 ppb. As with the other samples, seawater samples exhibited the highest variance for fluorescent materials in the range of optical brighteners. Overall, however, seawater samples showed the lowest variance for all dyes of interest.

Synchronous Scanning Protocol

As was done for field GAC samples, each grab sample was scanned using SSP to get a graphic representation of the sample and its constituents. Spectral graphs from each sample were examined to determine whether intensity values measured at a given wavelength during MWA could be attributed to a particular dye or background noise. Each graph was studied to find peaks which corresponded to the known excitation wavelengths of each dye. Peaks associated with fluorescein were visible on graphs associated with the Ypao beach spring and Asma Fenas River sites.

Table 7. **Summary of aqueous concentrations of fluorescent materials of interest in grab samples collected from all monitoring locations (units expressed in µg/L, or ppb).**

	Optical Brightener	Sodium Fluorescein	Eosine Y	Rhodamine WT
max	240	79.8	71.2	39.6
min	8.49	0.50	<0.005	<0.007
mean	60.7	15.1	12.1	8.53
std. dev. (σ)	44.3	18.1	15.3	8.43
var.(σ^2)	1964	327	234	71.0

Table 8. **Summary of aqueous concentrations of fluorescent materials of interest in GAC seawater samples (units expressed in ng/g, or ppb).**

	Optical Brightener	Sodium Fluorescein	Eosine Y	Rhodamine WT
max	135	2.12	<0.005	0.50
min	70.3	1.87	<0.005	0.20
mean	110	2.00	<0.005	0.33
std. dev. (σ)	20.7	0.074	24.0	0.079
var.(σ^2)	430	0.005	574	0.006

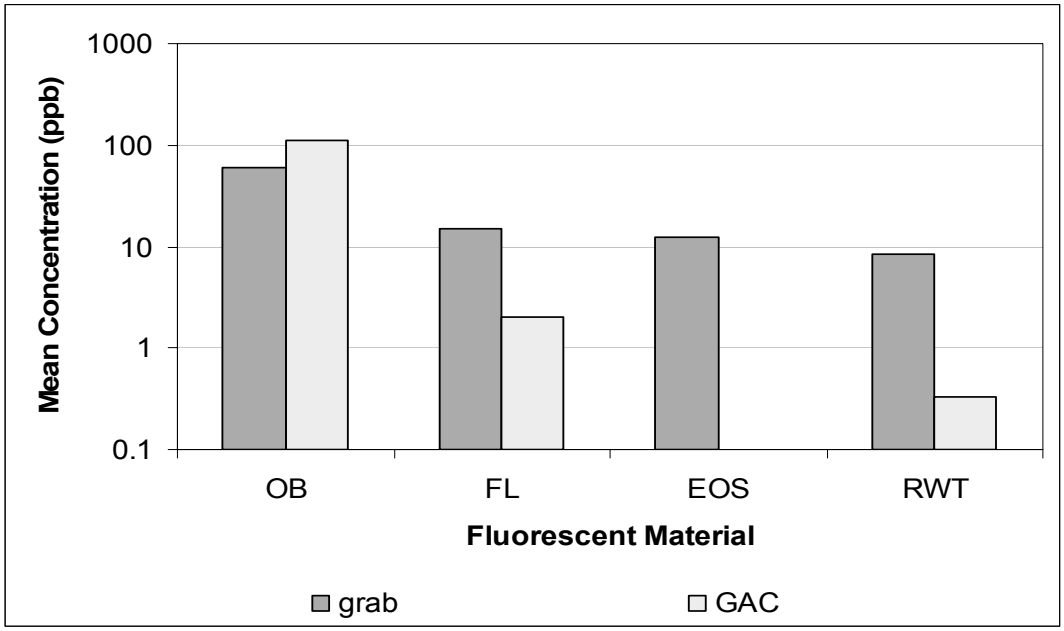

Figure 18. **Comparison of means for grab freshwater and GAC seawater samples.**

Precipitation Correlation

Daily precipitation data during the period of study were collected from five rain gauges around the island: Tiyan, Oka Point, Mangilao, Ipan and Mount LamLam. Island-wide rainfall data were normally distributed. Between January 2006 and April 2007, monthly rainfall (Figure 19) ranged from 1.12 to 57.48 cm, averaging 5.67 cm. Statistical analysis of rainfall data confirmed that the amount of rain at a given site for a given month is dependent on the time of year and geographic location. In addition, water table elevation data from May 2004 to February 2005 revealed aquifer responsiveness to rainfall events (Wuerch *et al.*, 2007[in press]).

Average monthly concentration data were plotted against total monthly rainfall (Hoffman, 2007). In addition, R^2 values were graphed to determine which sites corresponded most closely with which rain gauges. Seven of eight sampling sites exhibited an inverse relationship between precipitation and concentration for optical brighteners, indicating a dilution effect. An exception was the Asma Fenas River, which showed a weakly positive ($R^2 = 0.279$) power correlation between optical brightener levels and rainfall. At most sites, concentrations for compounds fluorometrically similar to fluorescein and rhodamine were positively correlated with rainfall. Exceptions to this trend included Dungca's Spring and Ypao Beach Spring for both dyes, Pago Spring for fluorescein, and Togcha Spring for rhodamine. Also, concentrations of compounds fluorometrically similar to fluorescein and rhodamine at the Asma Fenas River decreased with rain above 35 cm, exhibiting a strong, polynomial relationship (*e.g.*, $R^2 > 0.92$) with precipitation and indicating a dilution effect.

Nearly every site showed similar correlations (positive or negative) for both dyes, although each site was not necessarily similar to another. Interestingly, though, Pago Spring and Togcha Spring demonstrated opposite relationships for fluorescein and rhodamine and to one another. These two sites are not typically sampled during dye trace studies, and their recharge (catchment) areas are uncertain. It is not clear why these two sites do not follow the same trend as the rest of the sampling sites, but it is a noteworthy relationship.

In addition, most sites sampled showed the strongest relationship between concentration and precipitation at the nearest observed rain gauge. The two sites in East Agana Bay both corresponded most highly with the PCR rain gauge located about 1.7 km to the north on Oka Point. Concentrations at sites sampled in Tumon Bay tended to follow the precipitation observed at the NWS rain gauge in Tiyan, roughly 3.5 km to the south. Collectively, the sites along the east coast corresponded most closely with rain values in Tiyan, although individually the Togcha site corresponded most closely with rain at Ipan, approximately 0.4 km to the south.

Two sites were notable exceptions: Pago Bay and Asma Fenas River. Concentrations at the Pago Bay site more closely tracked the rain patterns observed at the Ipan gauge, located nearly 6.4 km to the south, instead of the Guam Community College's gauge, located just 3.5 km to the northeast in Mangilao. It was assumed that concentration data from the Asma Fenas River would be most closely associated with rainfall data obtained from a gauge located at the crest of its watershed. Instead, it tracked more closely with precipitation data from Ipan, Mangilao and Oka Point, in that order.

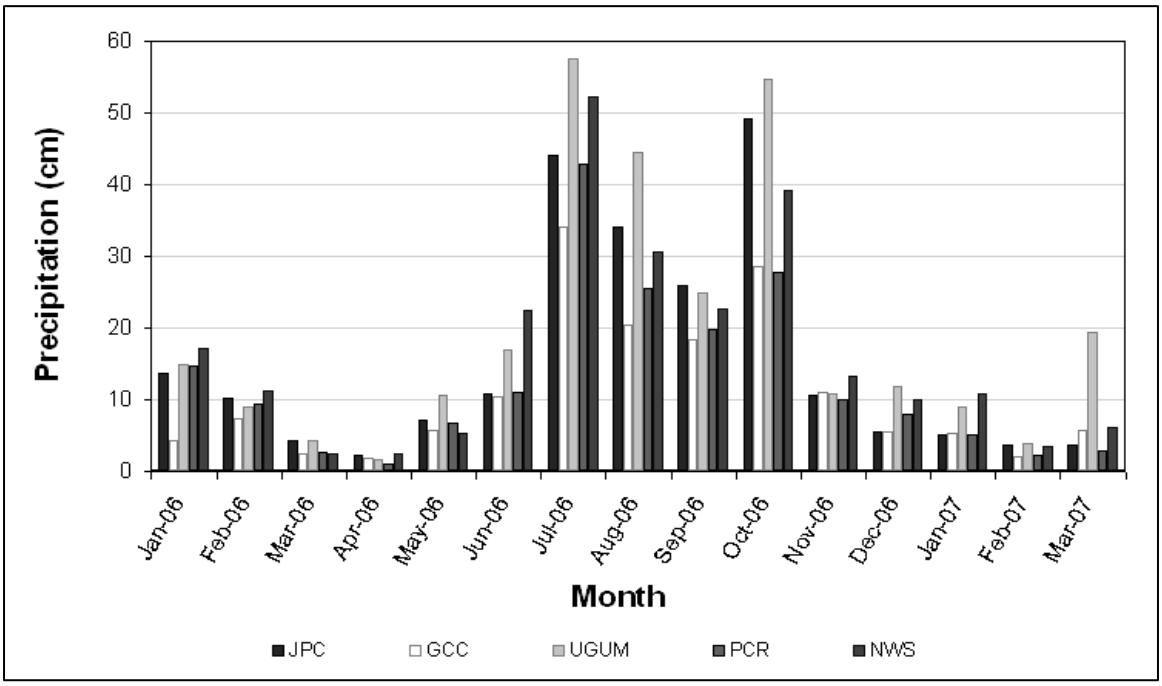

Figure 19. Total monthly rainfall during the period of study. Data sources: Jeff's Pirates Cove (JPC), D. Moran (GCC), WERI (UGUM), PCR Environmental (PCR), and National Weather Service (NWS).

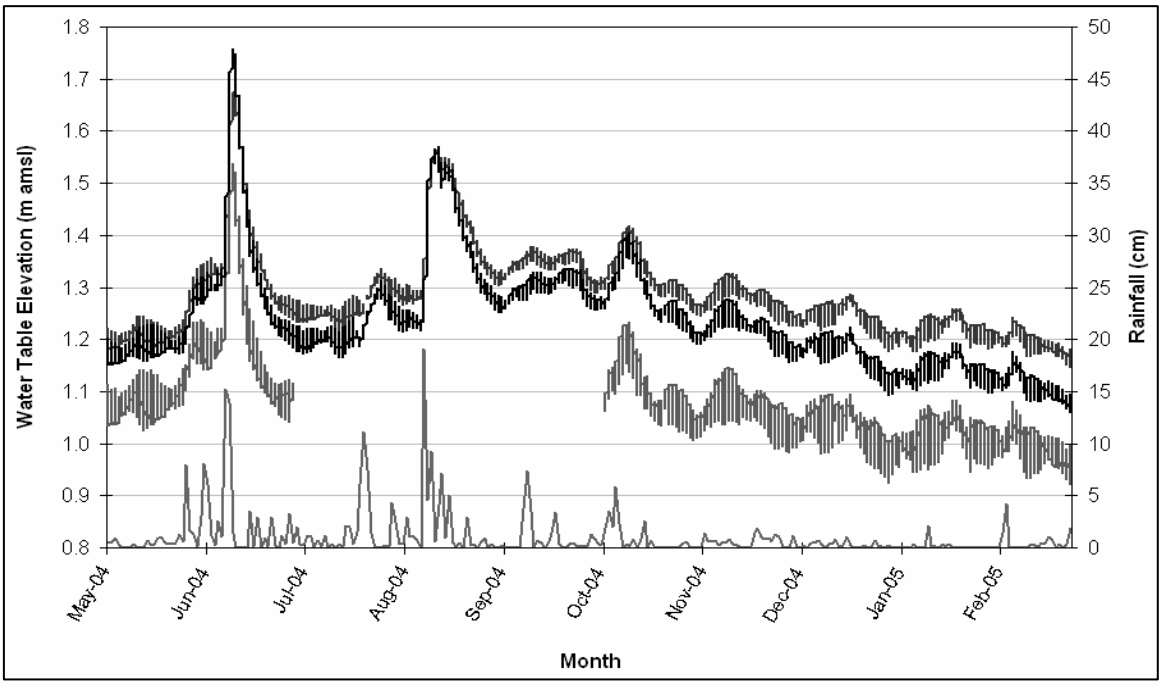

Figure 20. Aquifer response to rainfall as measured at three wells in the Yigo-Tumon Trough over a 9-month period. Source: Wuerch *et al.* (2007[in press]). Top 3 lines represent well level data. Bottom line indicates precipitation.

Other noteworthy observations include (1) the overall decrease in variability of dye concentrations with increase in total monthly rainfall, and (2) the seasonal pattern of optical brightener concentrations. With respect to the former, the greatest variance in concentration (islandwide) occurs when total monthly rainfall is less than approximately 15 cm, *i.e.*, during the dry season (June through November). With respect to the latter, equivalent optical brightener concentrations at most sites deviate below the mean during the dry season (roughly December through May), and above the mean during the rainy season (roughly June though November).

These findings suggest that surface runoff, and not submarine groundwater discharge, has the greatest influence on background levels. In addition, accurate detection of discharging dyes will be hampered during the dry season. As such, dye traces on Guam and similar tropical karst environments should be designed to encompass six months or less during the wet season. Dyes should be injected after the start of the rainy season, once the aquifer has been "primed", and monitoring should terminate before the following dry season commences. In cases when a dye trace must span more than six months, it may be advisable to have two sets of detection criteria, one for each season.

GENERAL CONCLUSIONS

Background concentrations of all four dyes of interest – optical brighteners, sodium fluorescein, eosine Y and rhodamine WT – vary significantly both in space and time. In addition, fluorescence in the range of optical brighteners consistently averages two orders of magnitude higher than the three xanthene dyes. This makes optical brighteners an uneconomical choice as a tracer on Guam, and likely also in any environment where dissolved organic compounds or wastewater effluent are present. Furthermore, data collected during this project indicate that fluorescein and rhodamine from previous injections continue to discharge from the aquifer in detectable quantities.

Fluorescent Organic Dyes as Groundwater Tracers

Essential properties of fluorescent dyes as tracers are their solubility and stability in water. High solubility is crucial because it allows for increased detectability despite substantial dilution in the environment. Stability is important to decrease the loss of tracer dyes to environmental factors (Field *et al*., 1995).

Tinopal and Phorwite (optical brighteners) have relatively high octanol-water partition coefficients -- log K_{ow} of 4.90 and 0.94, respectively (Field *et al*., 1995). This means that they have a stronger affinity for organic liquids (*e.g.*, lipids and solvents) than water. The octanol-water partition coefficient (K_{ow}) is directly related to the soil-water partition coefficient (K_{oc}), which reflects the affinity for a compound in aqueous solution for a carboniferous substrate (Connell, 1997). A large soil-water partition coefficient (K_{oc} >>1) means that a compound has a much stronger affinity for organic substrates than water and therefore is strongly attracted to GAC. Based on these characteristics, most of the optical brighteners which pass over a GAC sampler suspended in water are likely captured. Other organic substrates and biota upstream, however, may interfere with the quantities that reach the GAC.

Sodium fluorescein, eosine Y and rhodamine WT (the xanthene dyes), on the other hand, have very low octanol-water partition coefficients -- log K_{ow} of -0.39, -1.33 and -1.33, respectively (Field *et al*., 1995) -- and therefore very small soil-water partition coefficients (K_{oc} <1). This means they are highly water soluble and therefore highly mobile in the environment, but also less readily adsorbed onto GAC than the optical brighteners.

Sources of Variability

Several factors affect variability of background fluorescence, not only in this project but also for other similar projects and dye trace studies. Many factors are environmental and may be uncontrollable or unpredictable. Some may be an artifact of sampling protocols, analytical procedures or reporting parameters. Still others are the result of errors made by the sample collector or analyst at some point along the sampling and analysis trail. In the following sections, an effort has been made to account for as many of these factors as possible.

In the Environment

Natural Fluorescence

Besides the fluorescein derivative found in antifreeze (coolant), a common environmental contaminant, several other naturally-occurring compounds fluoresce in the same spectral range as dyes frequently used as tracers. Consequently, these substances are serious considerations when conducting a dye trace study. Both water contaminated by mineral oil products (Käss, 1998) and riboflavin found in foodstuffs (Becker *et al.,* 2003) fluoresce in the same range as optical brighteners. Caffeine and extracts

from boiled vegetables in wastewater effluent (Aley, 1999), as well as lime-secreting algae in tuffaceous limestone aquifers (Käss, 1998), can interfere with fluorescein detection.

Compounds which fluoresce in the same range as optical brighteners are ubiquitous in the environment, competing for adsorption sites on GAC, obscuring the dye of interest, and making positive detection difficult. In addition, the xanthene dyes have a lower affinity for GAC than the optical brighteners, making elution more efficient. These characteristics combine to make the xanthenes a favorable choice as tracers over optical brighteners.

Another interesting consideration are green fluorescent proteins (GFPs) found in marine coelenterates such as jellyfish, coral polyps, anemones, *etc*. (Tsien, 1998). These proteins can interfere across the spectrum; several derivatives exist which fluoresce anywhere between 350 and 550 nm. It is uncertain whether GFPs should be considered a significant source of interference. Proteins are crucial nutrients to scavenging marine organisms. As such, despite their potential prevalence in the suspended solids of nearshore marine waters, GFPs may not be very persistent in aquatic environments (Raymundo, 2006).

Particulates

Organic or inorganic particulates [*e.g.*, clays and silts (Davis and DeWeist, 1966), or humic acids and colloidal ferric hydroxides (Käss, 1998)] suspended in groundwater can interfere with direct fluorometric analysis. This is primarily a consideration for grab water samples, not GAC samples, although improperly rinsed GAC samplers can introduce particulates and fines into an elutant. Particulates in a water sample can either absorb or deflect excitation beams, which in turn affect emission scans. One way to avoid this problem is to filter water samples through undyed fiber or glass filters, or to decant water after allowing particulates to settle.

Humic and Fulvic Acids

Dissolved organic compounds, such as humic and fulvic acids, in surface waters can interact with fluorescent organic compounds which fluoresce between 350 and 450 nm (Käss, 1998; Baker and Lamont-Black, 2001), resulting in reduced fluorescent intensity (Aboul-Kassim and Simoneit, 2001) during excitation scans. For example, “weak” positive detections require cautious interpretation, since the presence of algae or dissolved organics can be mistakenly interpreted as fluorescein (Mull *et al.*, 1988). Furthermore, dissolved humic substances in the aqueous environment can interact with a fluorescent dye and reduce its solubility (Aboul-Kassim and Simoneit, 2001), inhibiting GAC adsorption and solvent extraction.

Sampling Substrate

Another source of interference when interpreting dye trace results arises from the use of granular activated charcoal (GAC) receptors. This variable was chosen for in-depth analysis. GAC acts an adsorptive substrate for organic dye molecules. Different factors affect interaction mechanisms between the liquid phase (dye) and the solid phase (GAC), including interfacial tension, cosolvency, precipitation, pH, colloidal stability, functional groups and cation exchange capacity (Aboul-Kassim and Simoneit, 2001). These interaction mechanisms mean that the longer GAC receptors are left *in situ*, the less effectively they detect dyes with short wavelengths (*e.g.*, optical brighteners). Contrarywise, the longer GAC is left in contact with eluent, the more intensely compounds with longer wavelengths (*e.g.*, rhodamine) fluoresce during analysis. So although GAC makes an efficient and inexpensive detector media, the ubiquity of fluorescent compounds in the environment dictates caution when interpreting the spectra of elutants (Smart and Simpson, 2002).

Another consideration with regard to sampling substrate is the use of GAC to detect optical brighteners. The preferred substrate is undyed, cotton pads (Aley, 1999), which are analyzed under ultraviolet light for the presence of optical brighteners. A literature review did not reveal any studies

which either (1) used GAC to detect optical brighteners, or (2) studied the effectiveness of using GAC for those dyes.

Deaminoalkylation of Rhodamine

Removal of alkylated amine functional groups (deaminoalkylation) is another consideration when using rhodamine derivatives for tracer tests, although the causes of deaminoalkylation are not clear from the literature. Deaminoalkylation lowers the absorbance and fluorescence wavelengths and results in two degradation byproducts (referred to as DARWT) which interfere with or mimic fluorescein and eosine (Käss, 1998; Idstein and Ewers, 2002). These byproducts can form in the field in as little as four weeks and persist as long as 12 years. In addition, DARWT can be detected concurrently with the original compound (Idstein and Ewers, 2002).

In the Laboratory

Precautions were taken to minimize sampling and analytical error. Nonetheless, activities such as sample collection, analysis and reporting could have affected the variability or accuracy of the values reported herein. An effort has been made to identify and evaluate as many sources of error as possible in the following sections.

Sample Turbidity

Turbidity in a sample has different effects. On one hand, it can inhibit absorbance and therefore decrease fluorescence, leading to an underestimation of concentration or prohibiting detection completely. On the other hand, refraction, bending and scattering can cause a false positive result by producing a fluorometric signal (Käss, 1998). Turbidity was observed in several elutants. Seventeen (roughly 7%) samples contained very fine charcoal sediment ("fines"), while 21 (about 8%) samples contained what appeared to be colloids.

Charcoal fines sometimes became entrained during the transfer of an elutant into a cuvette, resulting in a decrease in fluorescent intensity of the sample. Whenever this occurred, the sample was allowed to settle in the cuvette before being re-analyzed. The intensity was always greater the second time. A relationship was found between samples containing fines and two sample batches, implying the fines were an artifact of processing.

Initially, colloidal samples were analyzed like all other samples. They were then earmarked and saved for re-analysis. No relationship was found between colloidal samples and sampling location or duration of sampler deployment. Rather, it appeared to be related to three sample batches, implying the colloids were an artifact of processing rather than the sampling environment. Little to no settling of colloids was observed even after a prolonged period of time. Although the affected samples could have been filtered using unbleached paper or glass fiber filters (Käss, 1998), it involved the added risk of dye absorption or contamination. Instead, samples containing colloids were centrifuged at >10,000 rpm (per Käss, 1998), and the supernatant elutant was decanted into a cuvette and reanalyzed. Again, the intensity obtained during the second analysis was higher than the first.

Sample Elution

A standard eluent recipe of 5% potassium hydroxide and 95% isopropanol-water (7:3) was used to elute dyes from GAC (after Quinlan, 1987). Although this composition can be used with all three of the xanthene dyes, it is the most efficient for eluting fluorescein (Mull *et al.*, 1988). Eosine and rhodamine are better eluted with slightly different solvent compositions. For example, Smart (1972) recommended using 30 mL of eluent -- consisting of 38% ammonium hydroxide, 43% 1-propanol, and 19% distilled water -- per bug for the analysis of rhodamine WT. Solvent composition affects extraction efficiency (Figure 11), although to what extent is uncertain. One implication, however, is that low concentrations of

injected dye could get missed. An experiment was conducted during the course of this project to determine the extraction efficiency of the eluent and procedures. Results indicated that less than 100% of the adsorbed dye was removed from GAC from a single elution, ranging from about 40% for eosine and rhodamine to about 75% for fluorescein. This finding reinforces the earlier claim that the eluent used in this study is better suited for fluorescein.

Contamination, Degradation and Mislabeling

All glassware was rinsed in between sample transfers, but the possibility of cross-contamination still exists. Also, samples were stored in amber glass vials to protect them from photodegradation, and refrigerated to prevent biodegradation. Degraded samples would exhibit decreased or shifted fluorescence, underestimating concentrations or misidentifying constituent dyes. Samples were logged using an index system of 001 through 130, and so labeled for analysis to avoid bias. No evidence exists to suggest that any samples were inadvertently switched. This would not have affected analytical results, but would have affected variability estimates for a given location or sampling round.

Data Entry and Conversions

Spreadsheet software was used to enter raw intensity values obtained from the spectrofluorometer. This software was also used to convert intensity values to concentrations. Data and cell formula entries were double-checked against the analytical reports on two occasions, once in December 2006 and again in June 2007. Errors were few, and those found were corrected. Any mistakes made when manually recording the masses of each GAC receptor, would affect final estimations of concentration and estimates of variability between bugs.

Revisiting Recent Dye Trace Studies on Guam

Hoffman (2007) summarized the 1992 Air Force landfill study (AAFBER, 1995), the 2000 Harmon Sink study (Moran and Jenson, 2004) and the 2004 Navy landfill study (Earth Tech, 2006[draft]). Based on observations and data acquired during the course of this project, it seems insufficient quantities of eosine were used in each of these studies than was necessary to obtain fluorescence intensities comparable to fluorescein and rhodamine. This may explain, at least in part, why eosine was not only rarely detected in these dye traces, but also rarely detected in this baseline study. Furthermore, the Moran and Navy studies shared three sampling sites with this baseline study. A discussion of comparisons between the three studies follows.

Comparison of Background Values

Table 9 summarizes the results of the 2000 Moran (Moran, 2002; Moran and Jenson, 2004) and 2004 Navy (Earth Tech, 2006[draft]) studies for Dungca's Spring, Dungca's Stream and Ypao Spring. Since Earth Tech's data was reported in µg/L rather than ng/g, and data on charcoal masses were not available, all values have been standardized to µg/L. The results from the baseline study tend to fall between each of the previous studies' data sets. Recall that background samples in the Moran study were collected during the wet season and Earth Tech samples during the dry season. Accordingly, Moran's mean background levels are consistently higher than the Earth Tech's, whose background data are also frequently reported as 'zero' rather than 'non-detect'.

Each of these two dye trace studies based subsequent positive detections on their calculated background concentrations. Moran's positive detections were simply too conservative, although considerably less conservative than Earth Tech's. Moran's background values were either equal to or greater than the annual, islandwide means of this baseline study. Earth Tech's detections, on the other

hand, were not only much too conservative, they were based on samples obtained from a population of data (*i.e.* the dry season) that varies widely.

Table 9. Comparison of aqueous background values obtained from 2000 and 2004 dye traces and 2006 baseline study.

		Mean Concentration (µg/L)		
Site	**Study**	**FL**	**EOS**	**RWT**
Dungca's Spring	Moran 2000	2.22	1.96	NA
	Navy 2004	0.00	0.00	1.30
	Hoffman 2006	0.85	<0.05	0.91
Dungca's Stream	Moran 2000	1.23	0.95	NA
	Navy 2004	0.87	0.00	0.07
	Hoffman 2006	1.32	<0.05	0.43
Hilton/Ypao Spring	Moran 2000	0.57	0.73	NA
	Navy 2004	0.00	0.00	1.18
	Hoffman 2006	0.58	<0.05	0.24

Note: NA = not analyzed

RECOMMENDATIONS

Based on the results of this project, the following is recommended:

1. Avoid the use of optical brighteners and sodium fluorescein as tracers. Background levels of the former are too high to economically overcome in a dye trace, whereas the latter is also ubiquitous as a contaminant in surface runoff.
2. Set less conservative positive detection criteria. With a narrower definition of background, it is not necessary to set minimum detection levels orders of magnitude greater than background. Standard deviations of each dye should be considered, instead, and used to determine whether a given dye detection qualifies as a positive hit.
3. Conduct traces during the wet season whenever possible. Greater variability occurs during the dry season, and background samples collected during this time may greatly overestimate or underestimate background concentrations. During the wet season, however, background levels remain fairly stable.
4. Establish seasonal positive detection criteria. If a trace must span more than one season, then it is advisable to revisit positive detection criteria to be used as one season changes to the next.
5. Sample and correct for natural fluorescence levels in surrounding nearshore seawater. Nearly all of the monitored points of discharge are submerged in seawater, and GAC does not discriminate between freshwater discharge and seawater when adsorbing fluorescent organic compounds. Levels in the seawater must be subtracted from sample results.
6. Allow for variability associated with sampling substrate. Results from replicate sampling can vary by much as 94%. The longer samplers are left *in situ*, the greater the chance for losses to occur. Modify sampling schedules to minimize deployment intervals.

7. Use dye-appropriate solvents for extraction. On Guam, most samplers are sent off-island for analysis, and solvent selection is not available. This study showed that the standard recipe more efficiently extracts fluorescein than either eosine or rhodamine. If using these dyes and sending them away for analysis, inform the laboratory which dyes to analyze for and send enough sampling substrate to allow for subsampling using different solvents. This will increase positive dye detections.
8. Investigate the suitability of alternative tracing materials, such as tritium (Mink and Lau, 1977), helium (Carter *et al.*, 1959; Cădere, 1963) or spores (Smart and Smith, 1976; Käss, 1998). Gases such as tritium or helium have the benefit of being inert, colorless and easily detectable using spectroscopic methods. The use of *Lycopodium* spores requires less frequent sampling than using fluorescent dyes, although laboratory analysis is more time-consuming. Spores also provide quantitative rather than qualitative data that is unaffected by water chemistry and pollutants.

Suggestions for Future Research

Based on the questions and points of interest raised during the course of this study, the following studies are suggested:

1. Sample elution methods – For more accurate dye detection, especially for low concentrations, the appropriate solvent composition should be used depending on the dye of interest. Various eluent compositions should be investigated to determine which is the most efficient for which dyes, and which is the most economical for use with multiple dyes. In addition, elution methods should be tested to determine the most efficient means of extracting the highest yield of adsorbed dyes from charcoal.
2. Precipitation - A study should be conducted which addresses precipitation as a factor affecting the variability of coastal discharge (and, therefore, background fluorescence) over space and time.
3. Sampling frequency and duration – This project only addressed long-term variability on the higher end of sampling frequency, during a year flowing El Niño. Most dye traces begin with frequent sampling intervals on the order of hours, gradually increasing the intervals to days and weeks. A study should be performed in which one or more locations are sampled on a more frequent basis for a year, to encompass both the wet and dry seasons. Also, due to the year-to-year fluctuations of precipitation on tropical islands, this study should be duplicated for a second year to compare results long-term.
4. Green fluorescent proteins – An investigation into biotic sources of fluorescence in the nearshore marine environment should be initiated to determine the extent to which GFPs and similar metabolites influence background fluorescence at dye trace monitoring locations.
5. Inland wells – A similar baseline study which targets inland wells in the aquifer should be performed. This would remove the influence of ambient seawater and its constituents from the catalog of variables affecting background fluorescence.

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GLOSSARY

Adsorption
Attraction and adhesion of a layer of ions from an aqueous solution to the solid mineral surfaces with which it is in contact.

Anisotropic
Describes a geologic unit in which hydraulic properties of the aquifer differ spatially.

Aquifer
Rock or sediment in a formation, group of formations, or part of a formation that is saturated and sufficiently permeable to transmit economic quantities of water to wells and springs.

Average linear velocity
Rate of movement of fluid particles through porous media along a line from one point to another.

Colloids
Small particles dispersed in a liquid or gas phase; the liquid and solid forms of aerosols, foams, emulsions, and suspensions within the colloidal size class, typically 0.001 micron (μm) to 1 μm in any dimension.

Discharge
Volume of water flowing in a channel or aquifer past a specific point over a given period of time.

Dissolution
Process by which a solid is dispersed homogeneously into a liquid solution.

Eluent
Solvent used to extract fluorescent compounds from GAC; composed of isopropanol, water and potassium hydroxide.

Elutant
Solution of fluorescent compounds extracted from GAC using eluent.

Flank margin cave
Type of cave found on outer edges of tropical carbonate islands, typically formed by dissolution caused by the mixing of fresh and salt water at the edge of the freshwater lens, exhibiting rates of dissolution up to 1 m^3/yr; indicators of past sea levels throughout geologic history.

Fluoresce
To emit light at a longer wavelength when exposed to light of a shorter wavelength (see **Stokes shift**).

GAC
Granular, activated charcoal; can be made from coconut husks, wood, or a variety of other carbon-based products.

Heterogeneous
Describes a geologic unit composed of geologically and hydraulically dissimilar parts having nonuniform structure or composition.

Isotropic
Describes a geologic unit in which hydraulic properties of the aquifer are equal in all directions. ***Antonym: anisotropic.***

Karst
Type of geologic terrane underlain by carbonate rocks where significant solution of the rock has occurrence due to flowing groundwater.

Phreatic zone
Portion of the aquifer in which all the interstices are filled with water under pressure greater than that of the atmosphere; located below the **vadose zone**; also called the "saturated zone".

Permeability
Ability to transmit a fluid through a porous medium (such as rock or soil).

Recharge
Source of fresh water input, usually rain, into aquifer by soil infiltration or sinkholes.

Stokes shift
The difference in wavelength (or frequency units) between positions of the band maxima of the absorption and emission spectra of a fluorescent compound; both absorption and emission of energy are unique characteristics of a particular molecule during the fluorescence process.

Vadose zone
Subsurface zone between land surface and water table that includes root zone, intermediate zone, and capillary fringe; pore spaces contain water at less than atmospheric pressure, as well as air and other gases; also called "zone of aeration" and "unsaturated zone".

Water table
Upper surface of water in an unconfined aquifer; fluctuating boundary between vadose and phreatic zones; pore water pressure is atmospheric.

Xanthene
A compound $(C_6H_4)_2OCH_2$ (or dibenzpyran), from which xanthene dyes and other indicators are derived.