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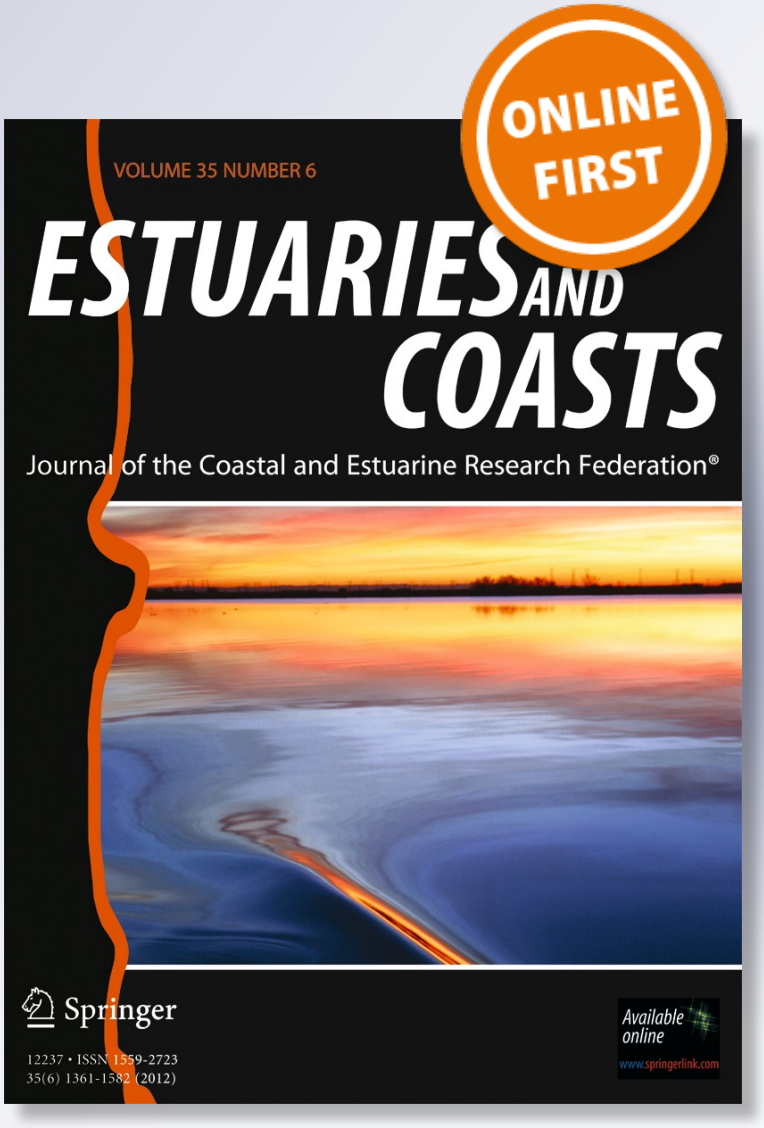

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A Comparison of Water Quality Between Low- and High-Flow River Conditions in a Tropical Estuary, Hilo Bay, Hawaii

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Abstract Effects of storms on the water quality of Hilo Bay, Hawaii, were examined by sampling surface waters at 6 stations 10 times during low-flow and 18 times during high-flow (storms) river conditions. The direction of a storm's impact on water quality parameters was consistent among storms and most stations; however, direction of the impact varied with the parameter. High river flow conditions increased concentrations of nitrate and decreased those of dissolved organic nitrogen (N); effects on ammonium and particulate N were station specific. Storms also increased dissolved organic and particulate carbon (C) concentrations. Dissolved phosphorus (P) concentrations were not affected by high river flow events. Dissolved organic forms dominated the N, C, and P pools under both low- and high-flow river conditions. Soil-derived particles and fecal indicator bacteria increased during storms, while chlorophyll *a* concentrations and bacterial cell abundances decreased. Our results suggest that an increase in storms with global warming could impact water quality of tropical estuaries.

Keywords Storms · Estuaries · Nutrients · Organic matter · Hawaii · Water quality

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Introduction

The number and intensity of storms are predicted to increase with global warming (Emanuel 2005; Webster et al. 2005). These storms bring heavy precipitation to watersheds causing flooding and rapid export of large quantities of watershed materials to coastal waters. Storms can deliver up to 80 % or more of the annual inputs of nutrients and particulates to estuaries (Eyre 1995; McKee et al. 2000; Wiegner et al. 2009), with some individual events accounting for 50 % of the annual inputs of these constituents within days (Paerl et al. 2001; Peierls et al. 2003). Consequently, these storm inputs affect chemical and biological parameters in estuaries including changes in water quality [nutrients, organic matter, fecal indicator bacteria (FIB)], dissolved oxygen concentrations, phytoplankton, zooplankton, fish, and benthic organism abundances and community compositions, as well as changes in prevalence of fish diseases (i.e., Mallin et al. 1999; Paerl et al. 2001; Mallin et al. 2002; Ringuet and MacKenzie 2005; Hoover et al. 2006). However, an individual storm can affect different estuaries differentially, and repeated storms to a particular system each may exert their own effects (Mallin et al. 2002; Paerl et al. 2006; Devlin and Schaffelke 2009). As a result, a large portion of storm research has been more event-based descriptions (i.e., De Carlo et al. 2007), rather than statistically quantitative comparisons among storms in a given

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estuary (Peierls et al. 2003; Williams et al. 2008; Paerl et al. 2009; Mead and Wiegner 2010).

Most of our knowledge about storm effects on estuaries is derived from studies of temperate estuaries along the east coast of the United States and subtropical estuaries along the east coast of Australia (i.e., Mallin et al. 1999; Paerl et al. 2001; Burkholder et al. 2004; Eyre and Twigg 1997; Eyre and Ferguson 2006). Impacts of storms on tropical estuaries are less well-known, with most research to date focusing on Kaneohe Bay, Oahu, Hawaii (Ringuet and MacKenzie 2005; Cox et al. 2006; Hoover et al. 2006; De Carlo et al. 2007), and a few estuaries in Australia, as well as coastal waters adjacent to the Great Barrier Reef (i.e., Eyre 1995; Eyre and Balls 1999; Devlin and Brodie 2005; Devlin and Schaffelke 2009; Brodie et al. 2010). In the tropics, seasonal changes in temperature and day length are relatively minor and have little effect on estuarine dynamics; instead, changes in rainfall are more important (reviewed in Eyre and Balls 1999). Therefore, storms play a greater role in affecting water quality and controlling primary and secondary production in tropical estuaries than seasonal climatic changes. In fact, abundance and diversity of coastal benthic communities are correlated with seasonal changes in rainfall and associated runoff in tropical systems (McCarthy et al. 2000; Restrepo et al. 2006). Tropical estuaries have been less studied than their temperate and subtropical counterparts because many of them are located in developing countries with limited resources; however, the need to better understand the natural dynamics and impacts of human activities on tropical coastal waters is imperative as one-third of the world's population lives in the tropics, with most living within 60 km of the coastline (Coughanowr 1998).

The following study focused on Hilo Bay, Hawaii, which is a tropical estuary greatly affected by storm pulses as it drains the windward sides the Earth's tallest and most massive mountains, Mauna Kea and Mauna Loa, respectively. The Hilo Bay watershed has one of the highest precipitation rates on the Hawaiian Islands and in the USA, with annual rainfall in this watershed ranging from 50 cm near shore to 600 cm at higher elevations (Juvik and Juvik 1998). Hence, the amount of freshwater entering Hilo Bay from surface flow and groundwater is greater than any other Hawaiian estuary. Additionally, the high slope, but relatively small size of Hawaiian watersheds compared to continental ones allows for quick fluvial responses to storms (Tomlinson and De Carlo 2003). Consequently, water quality changes in Hilo Bay should be rapid as the largest river in the state of Hawaii discharges into the bay, which is enclosed by a 3-km-long breakwater. Hilo Bay is a salt-wedge estuary that is stratified with a freshwater surface layer existing up to several kilometers offshore (Dudley and Hallacher 1991). There is minimal mixing between freshwater and saltwater layers inside the bay because the breakwater reduces wave energy, creating favorable conditions for phytoplankton blooms and trapping watershed-derived materials. Because of these characteristics, Hilo Bay is an ideal location to study storm effects on tropical estuarine water quality.

The question that our study addressed was: how does water quality in a tropical estuary immediately change following a storm? This question was addressed by sampling six stations within Hilo Bay during low and high river flow conditions. While most studies to date have reported day-to-day or month-to-month changes in estuarine water quality following storms (i.e., Valiela et al. 1998; Mallin et al. 1999; Ringuet and MacKenzie 2005; De Carlo et al. 2007), ours is one of the few that is statistically quantitative in examining the effects of multiple storms at multiple stations on estuarine water quality. To do this and make generalizations about how storms affect estuarine water quality, we designed our study and analyzed our data to look for differences in water quality between low and high river flow conditions. This following paper is one of three describing storm dynamics in Hilo Bay; the other two examine the biological response of Hilo Bay to the storm inputs in more detail, specifically with regards to surface water metabolism potential (Mead and Wiegner 2010) and food web dynamics (Atwood et al. 2011).

Materials and Methods

Site Description

Hilo Bay is located on the northeast side of Hawaii Island, Hawaii, USA. Approximately 9 km of the estuary's perimeter is bordered by land, while the outer margin is defined by a 3-km-long breakwater running east to west with a 1.5-km-wide opening to the Pacific Ocean (Fig. 1). Exchange of water through breakwater has been documented (M & E Pacific 1980). The partially enclosed bay has a nearly 6.4 km^2 surface area and ranges in depth from 0 to 15 m. Hilo Bay's watershed is the largest in the state of Hawaii (Juvik and Juvik 1998) and its surface water inputs are dominated by two rivers, the Wailuku River watershed to the north and the Wailoa River watershed to the south. The Wailuku River watershed is the largest watershed in the state of Hawaii, encompassing 576 km^2 with headwaters starting near 3,500 m in elevation on the slopes of Mauna Kea. The Wailoa River watershed encompasses 481 km^2 with headwaters starting near 762 m in elevation on the slopes of Mauna Loa. Both the Wailuku and Wailoa Rivers' watersheds are dominated by grasslands, evergreen forest, and scrub/shrub lands (average=27 %/50 %/13 %); however, the Wailoa River flows through a more anthropogenically impacted landscape compared to the Wailuku River, where ~15 % of its land use within the riparian zone is low- and high-intensity developed and cultivated lands (Mead and Wiegner 2010).

For this project, six stations were sampled for dissolved and particulate nutrients, chlorophyll *a* (Chl *a*), total bacteria

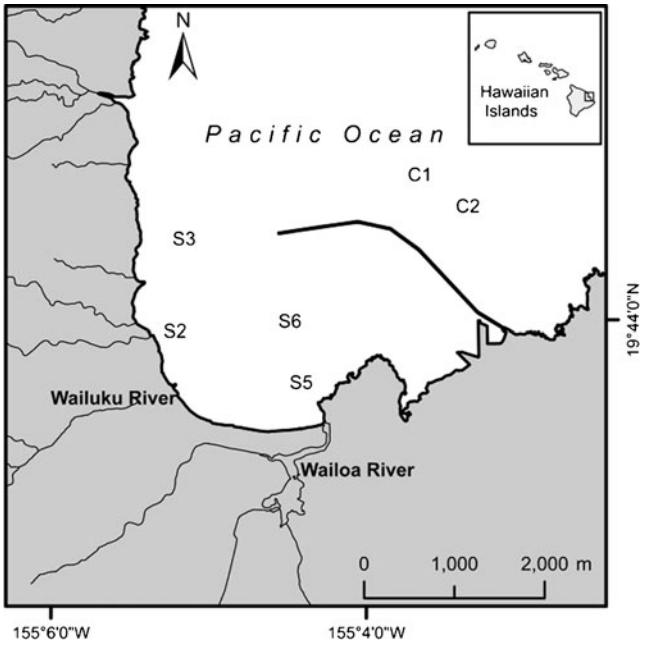

Fig. 1 Sampling stations in Hilo Bay, Hawaii, USA. *S* stands for station and *C* represents the stations that were used as controls. The controls were located outside of the breakwater and outside the direct influence of the Wailoa and Wailuku Rivers

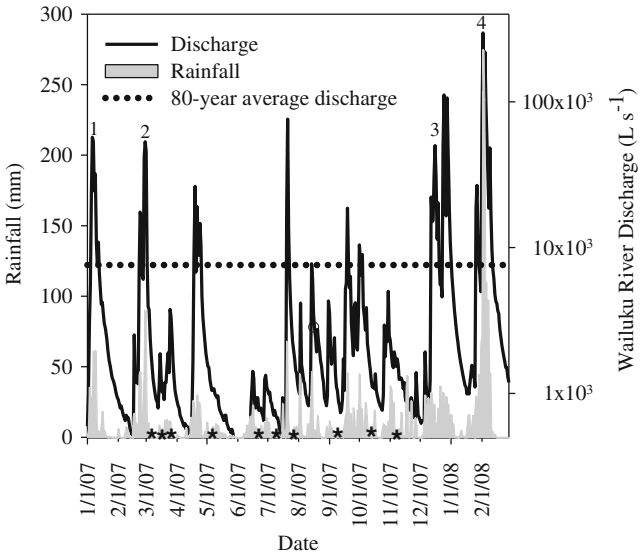

Fig. 2 Daily rainfall and discharge from the Wailuku River into Hilo Bay, Hawaii, USA, during study period. Rainfall data were for the Hilo International Airport and were obtained from the NOAA National Climatic Data Center. Discharge data for the Wailuku River were obtained online for the USGS gauge no. 16704000 and the 80-year average discharge for the river is shown (1929–2009). *Numbers on figure* indicate the four storms sampled for high river flow conditions (storm 1=5 days, storm 2=3 days, storm 3=5 days, storm 4=5 days; n=18 days total). *Asterisk* indicates days when low river flow conditions were sampled (n=10 days)

cell abundances, and FIB (Fig. 1). Four stations were located inside the bay, two along the Wailuku River plume (S2 and S3) and two along the Wailoa River plume (S5 and S6). Two "control" sites were chosen outside of the Hilo Bay breakwater in areas outside of the direct influence of the two rivers (C1 and C2) and are referred to as the Outer Bay stations in this paper. River water may, however, indirectly affect these Outer Bay stations, as the breakwater is permeable (M & E Pacific 1980). Latitude and longitude for all stations were recorded using a Garmin 2210C GPS receiver to ensure constancy of station locations among sampling dates.

Sampling Strategy

Water samples were collected from the bay stations 10 times during low-flow and 18 times during high-flow river (storms) conditions from January 2007 through February 2008. Low and high river flow categorization was based on daily discharge from the Wailuku River (USGS station no. 16704000), as it is the only gauged freshwater source into Hilo Bay. Low-flow conditions occurred when the Wailuku River's discharge was <2,500 Ls^{-1}, and high-flow events occurred when it was >3,500 Ls^{-1}. This designation was chosen because the lower perennial portion of the Wailoa River is hydrologically connected to its upper ephemeral portion when the Wailuku River's discharge is >3,500 Ls^{-1}. The Wailuku River's discharge generally increases with rainfall and so low-flow conditions occurred during dry periods of low rainfall and high-flow events occurred during storms (Fig. 2).

Sample Collection

Triplicate surface water samples from all stations were collected in a plastic bucket, pre-rinsed with sample water, poured into 1-L acid-washed high density polyethylene (HDPE) bottles, and immediately placed on ice during transport to the laboratory. Water samples for FIB analysis were collected in sterile containers that were immediately placed on ice during transport to the laboratory. Surface water samples were collected to assess effects of river flow on estuarine water quality because river sediments and phytoplankton concentrate at the surface due to density stratification (Dudley and Hallacher 1991). Additionally, temperature, salinity, and dissolved oxygen concentration were measured using a multi-parameter meter (YSI 85) at all stations. Their values are summarized in Table 1.

Sample Processing

At the laboratory, a known volume of water from the samples was filtered through pre-combusted (500 °C, 6 h), pre-weighed, GF/F filters (Whatman) and frozen until analysis for dissolved nutrients. The filters used here were then dried to a constant weight at 70 °C for total suspended solids (TSS), particulate carbon (PC), and particulate nitrogen (PN) determination. Additionally, a known volume of water was filtered through another filter which was stored frozen

Table 1 Average (± SE) values for physiochemical parameters measured in surface waters at six stations in Hilo Bay, Hawaii, USA, during low and high river flow conditions from 2007 to 2008

River flow	Station	*n*	Temp. (°C)	Salinity	pH	D.O. (mgL^{-1})
Low	S2	10	24.92±0.38	27.61±0.91	8.07±0.04	6.25±0.17
	S3	10	24.63±0.26	30.40±0.74	8.11±0.03	6.40±0.13
	S5	10	25.03±0.40	24.73±1.20	7.90±0.06	6.18±0.20
	S6	10	24.57±0.43	26.38±0.49	8.13±0.04	6.41±0.91
	C1	10	25.30±0.31	34.12±0.17	8.20±0.02	6.11±0.34
	C2	10	24.51±0.54	34.46±0.16	8.17±0.02	5.94±0.12
High	S2	17	22.15±0.27	22.05±1.23	8.12±0.04[a]	7.06±0.16
	S3	18	22.20±0.24	23.61±0.86	8.19±0.03	6.72±0.12
	S5	18	22.12±0.20	18.89±0.94	7.85±0.07	6.29±0.10
	S6	18	22.04±0.20	21.51±0.95	8.05±0.05	6.63±0.14
	C1	8	23.56±0.10	32.78±0.54	8.19±0.05[b]	6.03±0.09
	C2	8	23.15±0.16	33.20±0.46	8.21±0.04[b]	5.94±0.09

Temp. temperature, *D.O.* dissolved oxygen

[a] $n=18$

[b] $n=10$

in the dark for Chl *a* analysis. Aliquots of water from each sample were allowed to reach room temperature, and were analyzed for pH (Hanna HI 991301) and turbidity (Hach 2100P Turbidimeter).

Analytical Methods

Filtered nutrient samples were analyzed for total dissolved nitrogen (TDN), nitrate plus nitrite ($NO_3^-+NO_2^-$), ammonium (NH_4^+), total dissolved phosphorus (TDP), phosphate (PO_4^{3-}), silicic acid (H_4SiO_4), and dissolved organic carbon (DOC). $NO_3^-+NO_2^-$ [USEPA 353.4, detection limit (d.l.) 0.1 $\mu molL^{-1}$], NH_4^+ [USGS I-2525, d.l. 1 $\mu molL^{-1}$], TDP (USGS I-4650-03, d.l. 0.1 $\mu molL^{-1}$), PO_4^{3-} (USEPA 365.5, d.l. 0.1 $\mu molL^{-1}$), and H_4SiO_4 (USEPA 366, d.l. 5 $\mu molL^{-1}$) were analyzed on a Technicon Pulse II Autoanalyzer. Dissolved organic phosphorus (DOP) was determined from the difference between TDP and PO_4^{3-}. TDN was analyzed by high-temperature combustion, followed by chemiluminescent detection of nitric oxide (Shimadzu TOC-V, TNM-1, d.l. 5 $\mu molL^{-1}$). Dissolved organic nitrogen (DON) was determined from the difference between TDN and dissolved inorganic nitrogen ($DIN=NH_4^++NO_3^-+NO_2^-$). DOC was measured by high-temperature combustion (Shimadzu TOC-V, TNM-1, d.l. 10 $\mu molL^{-1}$) following recommendations of Sharp et al. (2002). All nutrient samples were analyzed within two weeks of collection. Dried filters were reweighed for TSS determination (APHA et al. 1995) and subsequently analyzed for PC and PN on a CHN analyzer (Costech Analytical Technologies). All dissolved and particulate nutrient samples were analyzed at the University of Hawaii at Hilo Analytical Laboratory. Frozen filters were processed according to USEPA method 445.0 for Chl *a* and analyzed on a Turner 10-AU fluorometer. Total bacteria cell abundances were determined on pre-filtered (0.6-μm; Nucleopore polycarbonate track-etch membranes) sub-samples (20 to 60 μL) diluted with sterile filtered (Nucleopore polycarbonate track-etch membranes) deionized water, stained with three drops of 100 ug mL^{-1} 4′6-diamidino-phenylindole (DAPI, Sigma-Aldrich) for three to five min. The sub-samples were then filtered onto black 0.2-μm filters (Nucleopore polycarbonate track-etch membranes), which were placed on slides and analyzed using an Olympus BX51 epifluorescent microscope with an ultraviolet filter at 100× magnification (method modified from Porter and Feig 1980). Ten fields per slide were counted, averaging (±SE) 61±2 bacteria per sample.

The FIB *Enterococci* were isolated on mEI agar according to USEPA Method 1600, incubated at 41 °C, and colony forming units (CFU) were enumerated after 24 h. Membrane filters that contained approximately 50 or more CFU were analyzed for the presence of human-specific genetic markers, the enterococcal surface protein gene (*esp*) in *Enterococcus faecium* and *Enterococcus faecalis* using primers specific to bacteria of human origin (Shankar et al. 1999; Scott et al. 2005). Bacterial colonies were removed from the membrane filters by suspending filters in 10 mL of tryptic soy broth and incubating for 2 h at 41 °C. DNA was extracted from 1 mL of this suspension using the Qiagen QIAamp DNA Mini Kit according to the manufacturer's directions (cell lysis through DNA purification). The forward primer, which is specific for the *E. faecium esp* gene, used was: 5′-TAT GAA AGC AAC AGC ACA AGT T-3′ (Scott et al. 2005), and the conserved reverse primer used was: 5′-ACG TCG AAA GTT CGA TTT CC-3′ (Hammerum and Jensen 2002). The forward primer, which is specific for the *E. faecium*/*E. faecalis esp* gene, used

was: 5′-TTG CTA ATG CTA GTC CAC GAC C-3′; the reverse primer sequence used was: 5′-GCG TCA ACA CTT GCA TTG CCG AA-3′ (Shankar et al. 1999). PCR reactions contained 1× PCR buffer, 1.5 mmolL^{-1} $MgCl_2$, 200 μmolL^{-1} of each dNTP, 0.3 μmolL^{-1} of each primer, 0.5 U of HotStarTaq DNA polymerase (Qiagen), and 1 μL template DNA per 20 μL reaction. Amplification was performed with an initial step at 95 °C for 15 min, followed by 35 cycles of 94 °C 1 min, 58 °C 1 min, 72 °C 1 min, with a final extension at 72 °C 7 min. PCR products were separated on a 1.5 % agarose gel stained with GelStar nucleic acid stain (BioWhittaker) and viewed under ultraviolet light.

Statistical Analyses

As mentioned earlier, three independent water samples were collected at each station on each sampling date. Daily values for the three water samples were averaged and the averages were analyzed statistically. Differences in concentrations for dissolved nutrients, particulates, and biological parameters, as well as N, C, and P pools' compositions were examined by two-way Analysis of Variance (ANOVA) with region (Wailuku River plume, Wailoa River plume, Outer Bay) and river flow condition (low- vs. high-flow) as factors. Data that did not satisfy normality and equal variance requirements for ANOVA were transformed [log, natural log, square-root, rank (Potvin and Roff 1993)] prior to ANOVA analyses or analyzed using a Kruskal-Wallis test (DON only). Significant ANOVA results (α=0.05) were further analyzed using the Tukey HSD multiple comparison test. Correlation and linear regression analyses were also used to examine and determine relationships among variables. All statistics were run using Systat® 11. Results from statistical analyses are shown below in parentheses with p values provided.

Results

Nitrogen

Concentrations of all constituents in the N pool significantly differed between low- and high-flow river conditions (Fig. 3, Table 2). NO_3^-+NO_2^- concentrations increased during high river flow within all regions of Hilo Bay (p<0.001), and significantly differed among regions (p<0.001), with the highest concentrations measured within the Wailoa River plume, followed by the Wailuku River plume, and the Outer Bay. In contrast to NO_3^-+NO_2^-, there was a significant interaction between river flow condition and region for NH_4^+ (p=0.020), where NH_4^+ concentrations decreased during high river flow conditions within the two river plumes and increased in the Outer Bay. PN also had a significant interaction between river flow conditions and region (p=0.018), where PN increased in the Wailuku River plume during high river flow conditions, but remained the same within the Wailoa River plume and Outer Bay. DON concentrations decreased during high-flow events (p=0.032) and significantly varied among regions (p=0.004), with the Outer Bay and Wailuku River plume having higher concentrations than the Wailoa River plume (Fig. 3, Table 2).

Across most regions sampled in Hilo Bay, the dominant form of N during both low- and high-flow river conditions was DON, comprising (average ± SE) 53 %±3 and 43 %±2 of the N pool, respectively (Table 3). DON's contribution to the N pool also significantly decreased (p<0.001) by ~10 % from low- to high-flow river conditions and differed among regions (p<0.001), with DON comprising the greatest percentage of the N pool within the Outer Bay followed by the Wailuku River plume, and then the Wailoa River plume (Table 3). The contribution of NO_3^-+NO_2^- to the N pool in contrast to DON increased by ~9 % from low- to high-flow river conditions within all regions (p<0.001) and was highest within the Wailoa River plume (p<0.001), comprising approximately 50 %±3 of the N (Table 3). For NH_4^+, there was a significant interaction between river flow condition and region (p=0.027), where NH_4^+'s contribution to the N pool increased from low- to high-flow river conditions within the Wailuku River plume and Outer Bay, and decreased within the Wailoa River plume (Table 3). In contrast to the other three N parameters, PN's contribution to the N pool in Hilo Bay was not affected by river flow condition (p=0.144), but did differ among regions (p=0.006), with PN comprising a much larger percentage of the N pool within the Wailuku River plume compared to the Wailoa River plume and Outer Bay (Table 3).

Carbon

DOC and PC concentrations both differed between river flow conditions (p<0.001) and among regions (p≤0.003; Fig. 3, Table 2). DOC concentrations increased within the Wailuku and Wailoa River plumes during storms, but remained fairly constant between river flow conditions within the Outer Bay (Fig. 3). DOC concentrations were ~20 μmolL^{-1} higher within the Wailuku River plume compared to the Wailoa River plume and Outer Bay regions across river flow conditions (Fig. 3). PC also increased during storms within all three regions in Hilo Bay, with the greatest increase observed within the Wailuku River plume, where the PC concentration more than tripled during storms (Fig. 3, Table 2). The two river plumes had PC concentrations two to three times higher than concentrations measured within the Outer Bay across river flow conditions (Fig. 3, Table 2).

Across all regions sampled in Hilo Bay, the dominant form of organic C during both low- and high-river flow conditions

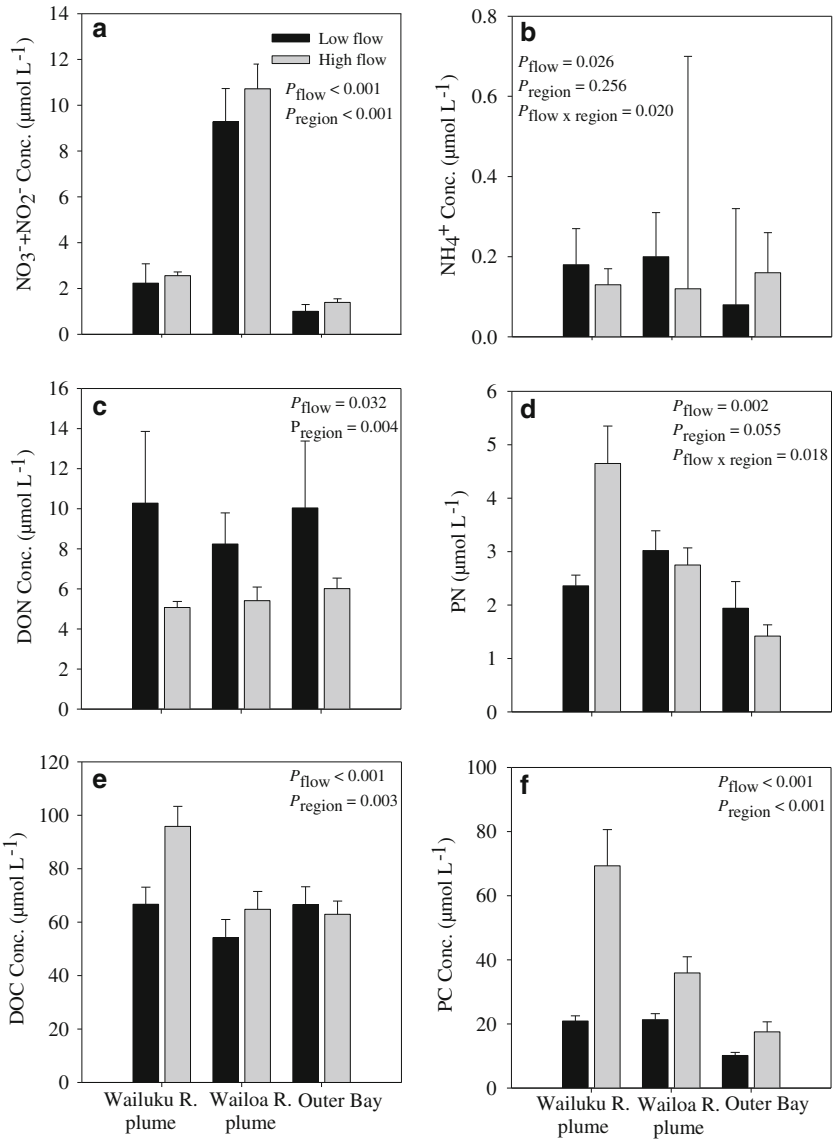

Fig. 3 Comparison of average(± SE) surface water (**a**) $NO_3^-+NO_2^-$, (**b**) NH_4^+, (**c**) dissolved organic nitrogen (DON), (**d**) particulate nitrogen (PN), (**e**) dissolved organic carbon (DOC), and (**f**) particulate carbon (PC) concentrations under low and high river flow conditions in the three regions examined in Hilo Bay, Hawaii, USA, from 2007 to 2008. Results from two-way ANOVAs and Kruskal-Wallis tests are shown on figure (α=0.05)

was DOC, comprising 74 %±2 and 66 %±2, respectively (Table 3). DOC's contribution to the organic C pool decreased slightly (~8 %; p=0.055) from low- to high-flow conditions and was similar among regions (p=0.061; Table 3). In contrast to DOC, PC's contribution to the organic C pool increased slightly from low- to high-flow (p=0.055) and also was similar among regions (p=0.061; Table 3).

Phosphorus

Of the samples collected during our study, only 22 %, 20 %, and 7 % had detectable TDP, DOP, and PO_4^{3-} concentrations. When these constituents were present in detectable concentrations, TDP and DOP concentrations were similar between river flow conditions and among regions, averaging 0.10± 0.02 µmol L^{-1} and 0.09±0.02 µmol L^{-1}, respectively. PO_4^{3-} concentrations were similar between river flow conditions, but different among regions (p=0.025). The only region where PO_4^{3-} concentrations were detectable was within the Wailoa River plume. The relative contributions of PO_4^{3-} and DOP to the TDP pool were similar between river flow conditions (p= 0.563) and among regions (p=0.483) in Hilo Bay. On average, PO_4^{3-} and DOP contributed 11 %±0.05 and 89 %±0.05 to the P pool, respectively.

Table 2 Average (± SE) nutrient concentrations, total suspended solids, turbidity, chlorophyll *a* values, total bacteria cell abundances, and *Enterococcus* levels in surface waters at six stations in Hilo Bay, Hawaii, USA, during low (n=10) and high river flow (n=18) conditions

River flow	Station	TDN (μmol NL^{-1})	$NO_3^-+NO_2^-$ (μmol NL^{-1})	NH_4^+ (μmol NL^{-1})	DON (μmol NL^{-1})	PN (μmol NL^{-1})	DOC (μmol C L^{-1})	PC (μmol C L^{-1})	DOP (μmol P L^{-1})	H_4SiO_4 (μmol Si L^{-1})	TSS (mgL^{-1})	Turb. (NTU)	Chl *a* (μgL^{-1})	Bac. Abund. (cells× 10^9L^{-1})	*Enterococcus* (CFU 100 mL^{-1})[a]
Low	S2	13±5	1.2±0.4	0.2±0.1	11±6	2.5±0.3	68±9	24.1±2.3	0.2±0.1	50±7	14.18±2.00	0.86±0.11	0.92±0.18	8.80±2.77	8.50±2.93
	S3	12±5	3.3±1.6	0.2±0.2	9±5	2.2±0.3	65±9	17.7±2.0	0.1±0.1	61±12	13.24±1.68	0.72±0.09	3.68±1.40	7.01±1.74	4.21±0.99
	S5	20±4	13.1±2.0	0.2±0.1	7±1	2.8±0.4	46±10	20.7±2.0	0.1±0.1	181±17	11.44±1.90	0.79±0.10	2.46±0.62	6.05±1.50	1.99±0.64
	S6	15±4	5.4±1.3	0.2±0.2	9±3	3.2±0.7	62±9	21.9±3.3	0.1±0.1	113±8	11.27±1.48	0.74±0.08	5.56±2.37	3.65±1.15	1.62±0.50
	C1	11±5	0.7±0.3	0.1±0.1	10±5	2.0±0.6	68±10	10.9±1.5	0.1±0.1	11±2	8.28±1.07	0.29±0.05	1.41±0.37	3.99±0.74	18.35±10.43
	C2	12±5	1.3±0.6	0.1±0.1	10±5	1.9±0.8	66±10	9.4±1.2	0.1±0.1	17±9	6.99±0.86	0.27±0.03	1.02±0.29	4.60±0.77	1.09±0.09
High	S2	8±0	2.4±0.2	0.1±0.1	5±0	5.5±1.2	100±12	85.5±19.0	0.1±0.1	46±4	28.95±1.94	5.96±1.43	0.13±0.07	4.01±0.57	362.40±81.26
	S3	8±0	2.7±0.3	0.1±0.1	5±1	3.8±0.7	92±10	53.0±14.0	0.0±0.0	47±4	26.82±2.93	3.93±0.93	0.33±0.14	3.91±0.76	289.48±0.99
	S5	20±1	14.8±1.5	0.1±0.1	5±1	2.4±0.2	48±7	28.8±3.0	0.1±0.0	173±19	14.69±1.73	2.14±0.31	0.35±0.11	3.96±0.49	208.61±63.82
	S6	13±1	6.7±0.8	0.2±0.1	6±1	3.1±0.6	82±10	43.0±9.5	0.0±0.0	95±12	21.57±1.77	3.40±0.82	0.30±0.12	3.91±0.46	245.20±67.01
	C1[b]	8±1	1.5±0.3	0.2±0.2	6±1	1.5±0.3	62±6	19.0±4.8	0.1±0.1	16±3	21.20±1.87	1.28±0.56	0.14±0.07	4.37±0.93	319.09±126.66
	C2[b]	8±1	1.3±0.2	0.2±0.1	6±1	1.3±0.3	64±8	16.0±4.2	0.0±0.0	13±3	22.77±2.18	1.07±0.47	0.39±0.14	4.48±0.78	140.06±130.81

TDP concentrations are not reported here as most TDP was DOP. PO_4^{3-} concentrations are not reported here as most measurements were below detection limits

TDN total dissolved nitrogen, *DON* dissolved organic nitrogen, *PN* particulate nitrogen, *DOC* dissolved organic carbon, *PC* particulate carbon, *TSS* total suspended solids, *Turb*. Turbidity, *Chl a* chlorophyll *a*, *Bac. Abund.* total bacteria cell abundances, *Enteroc*. *Enterococcus*, *TDP* total dissolved phosphorus, *DOP* dissolved organic phosphorus

[a] Low river flow: S3 n=4, S4 n=5, S5 n=4, S6 n=3, C1 n=5, and C2 n=3. High river flow: S3 n=10, S4 n=2, S5 n=10, S6 n=10, C1 n =2, and C2 n=2

[b] n=12

Table 3 Average (± SE) percentage contribution of N ($NO_3^-+NO_2^-$, NH_4^+, DON, PN) and C (DOC, PC) forms to the total N and C concentrations in surface waters at six stations in Hilo Bay, Hawaii, USA, during low (n=10) and high river flow (n=18) conditions

River flow	Station	$\%NO_3^-+NO_2^-$	$\%NH_4^+$	%DON	%PN	%DOC	%PC
Low	S2	10±3	2±1	63±5	25±4	70±6	30±6
	S3	22±9	2±2	52±9	23±5	73±6	27±6
	S5	59±7	1±1	25±8	15±3	65±4	36±4
	S6	30±7	2±1	44±6	25±6	71±5	29±5
	C1	8±3	1±1	71±5	20±5	82±5	18±5
	C2	15±6	2±2	64±7	20±6	83±4	17±4
High	S2	20±2	1±1	42±4	37±4	59±3	41±3
	S3	25±3	1±0	45±4	29±3	67±3	33±3
	S5	65±5	0±0	24±5	11±1	59±4	41±4
	S6	42±4	1±1	35±3	21±4	67±3	33±3
	C1	16±3	1 ±1	66±5	17±3	77±4	23±4
	C2	15±1	1±1	69±5	15±3	80±4	20±4

DON dissolved organic nitrogen, *PN* particulate nitrogen, *DOC* dissolved organic carbon, *PC* particulate carbon

Biological Parameters

Chl *a* significantly differed between river flow conditions ($p<0.001$), but was similar among regions in Hilo Bay. Chl *a* concentrations were an order of magnitude higher during low-flow conditions (2.51±0.51 $\mu g L^{-1}$) than during storms (0.27±0.05 $\mu g L^{-1}$; Fig. 4, Table 2). Likewise, total bacteria cell abundances decreased from low- ($5.89\pm0.66\times10^9$ cells L^{-1}) to high-flow river conditions ($4.05\pm0.26\times10^9$ cells L^{-1}; Fig. 4, Table 2); however, the decrease was not significant (p=0.066). Additionally, total bacterial cell abundances were similar among the regions sampled in Hilo Bay (p=0.455; Fig. 4, Table 2). In contrast, FIB *Enterococcus* levels significantly increased from low- (8.44±2.2 CFU 100 mL^{-1}) to high-flow conditions (294.00±44.3 CFU 100 mL^{-1}; $p<0.001$; Fig. 4, Table 2); however, they were similar among the regions sampled in Hilo Bay (p=0.206). All samples were negative for both human fecal pollution *esp* gene markers.

Discussion

Nitrogen

Like in Hilo Bay, elevated $NO_3^-+NO_2^-$ concentrations in estuarine waters have been observed following storms in subtropical and tropical estuaries in Australia, a tropical estuary in Hawaii, and temperate ones along the east coast of the United States (i.e., Ruzecki et al. 1977; Eyre and Balls 1999; Eyre 2000; Paerl et al. 2001; Ringuet and Mackenzie 2005). Higher $NO_3^-+NO_2^-$ concentrations in estuaries following storms generally result from elevated concentrations in rivers and greater river discharge, and have been attributed to increased leaching of materials from the watershed and river channel (Eyre and Twigg 1997). However, in Hilo Bay, $NO_3^-+NO_2^-$ concentrations in the Wailuku and Wailoa Rivers during storms were not elevated; in fact, concentrations in these rivers remained relatively constant or were slightly lower during storms (Wiegner and Mead 2009), a pattern previously documented for the Wailuku River (Wiegner et al. 2009). In contrast, instantaneous daily $NO_3^-+NO_2^-$ yields from these two rivers were greater during storms, especially from the Wailuku River, where they were almost two orders of magnitude greater than those under low-flow conditions (Wiegner and Mead 2009). While increased riverine $NO_3^-+NO_2^-$ yields may explain increased concentrations inside the breakwater, they may not fully explain the ones outside of it. The breakwater is permeable and it is likely that some of the riverine $NO_3^-+NO_2^-$ diffused through to the Outer Bay stations; however, the river plume itself flowed northwest along the coastline in the opposite direction of the Outer Bay stations. This latter observation and the consistent pattern of increased $NO_3^-+NO_2^-$ concentrations during high river flow in all three regions in the bay suggest that $NO_3^-+NO_2^-$ may have been produced within Hilo Bay, possibly through nitrification immediately following storms. Nitrification within turbidity maximum zones of northern European estuaries has been documented (Sebilo et al. 2006; Dähnke et al. 2008; Schlarbaum et al. 2010) and suggests that this phenomenon may also occur within turbidity plumes generated from storms.

Changes in NH_4^+ concentrations following storms have been consistently measured in estuaries and they have been observed to both increase and decrease following floods depending on the system and event (Mallin et al. 2002; Peierls et al. 2003; Cox et al. 2006; Eyre and Ferguson 2006; De Carlo et al. 2007). Increases have been attributed to release of NH_4^+ from the decay of organic matter in the

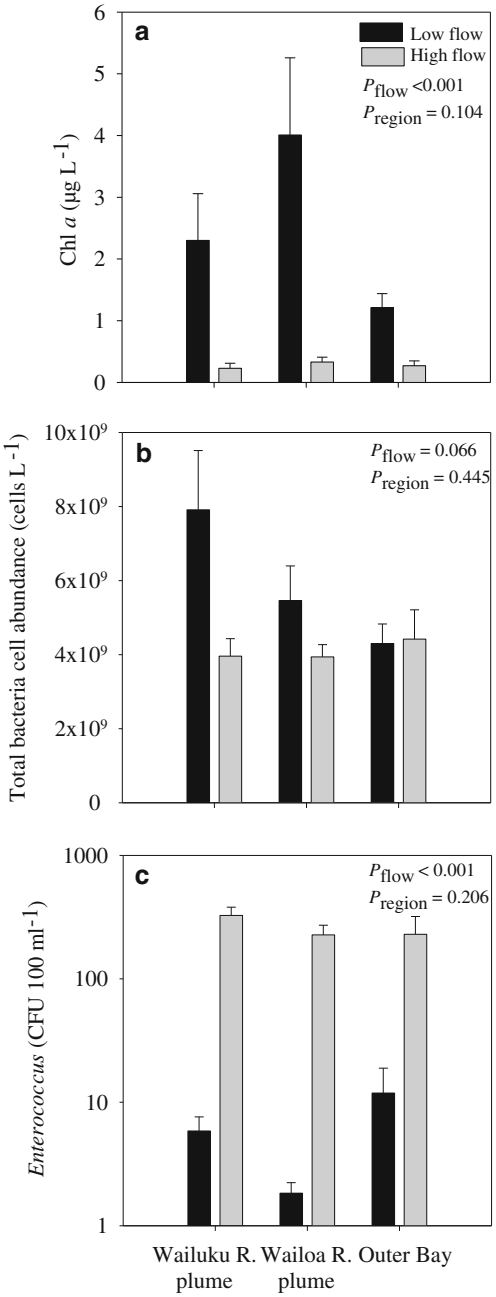

Fig. 4 Comparison of average (± SE) surface water (**a**) chlorophyll *a* (Chl *a*), (**b**) total bacteria cell abundance, and (**c**) *Enterococcus* values under high and low river flow conditions in the three regions examined in Hilo Bay, Hawaii, USA, from 2007 to 2008. Results from two-way ANOVAs are shown on figure (α=0.05)

water column and benthos (Valiela et al. 1998; Peierls et al. 2003) and decreases have been attributed to dilution of point sources into estuaries (Eyre and Ferguson 2006). There are currently no point sources of NH_4^+ in Hilo Bay, so dilution of a point source does not explain the decreases in NH_4^+ following storms. As suggested above, nitrification may have consumed some of the NH_4^+ in the water column, as NO_3^-+NO_2^- concentrations increased following storms.

In contrast to NH_4^+, few studies have measured PN in estuaries following storms (Cox et al. 2006; Eyre and Ferguson 2006; Devlin and Schaffelke 2009; Brodie et al. 2010). The two previous studies that compared low to high river flow conditions found that PN decreased in estuaries immediately following storms (Cox et al. 2006; Eyre and Ferguson 2006). These decreases in PN were attributed to phytoplankton biomass being diluted by watershed debris or flushed out of the estuary during storms, as PN was strongly correlated with Chl *a* concentrations in these systems (Cloern 1996; Eyre 2000; Peierls et al. 2003; Ferguson et al. 2004; Eyre and Ferguson 2006). In Hilo Bay, this was not the case, as the response of PN to storms was region-specific; it significantly increased in the Wailuku River plume and was unaffected in both the Wailoa River plume and Outer Bay (Fig. 3). Additionally, PN was not correlated to Chl *a* under these conditions, but was correlated to turbidity and PC suggesting PN was a constituent of the particles being flushed out of the watershed (Fig. 5). The C:N ratio of the particulate matter supports this supposition as the slope of a linear regression between PC and PN was 16 during storms, a ratio comparable to those found in Hawaiian soils (Crews et al. 1995), and 7 during low river flow conditions, a ratio indicative of plankton (Redfield et al. 1963; Parsons et al. 1961).

Like PN, DON concentrations in estuarine waters following storms have been measured in only a few systems, with a decrease in concentration following storms being the most common observation (Cox et al. 2006; Eyre and Ferguson 2006; De Carlo et al. 2007) and contrasting with reported increases in DOC concentrations (Paerl et al. 2001; Williams et al. 2008). Note, however, our study is the first to measure DON and DOC concentrations simultaneously in an estuary following a storm. The most likely reason for the observed decreases in DON relative to DOC in Hilo Bay during storms is that the majority of water volume in Hilo Bay during storms was comprised of water from the Wailuku River which had lower DON and higher DOC concentrations (Wiegner and Mead 2009). Another possible explanation for decreases in DON concentration is ammonification of DON and subsequent nitrification of NH_4^+ (Kerner and Spitzy 2001; Badr et al. 2008), as DON concentration decreases in all three regions in Hilo Bay could account for the observed NO_3^-+NO_2^- increases.

Of all of the forms of N measured in our study, DON was the dominant one across most stations sampled in Hilo Bay during both low- and high-flow river conditions, comprising 53 %±3 and 43 %±2, respectively. However, DON's contribution to the N pool decreased by ~10 % from low- to high-flow conditions and differed among regions. Differences among the regions in Hilo Bay can in part be explained by whether a region was directly affected by groundwater, river discharge, or ocean exchange. The

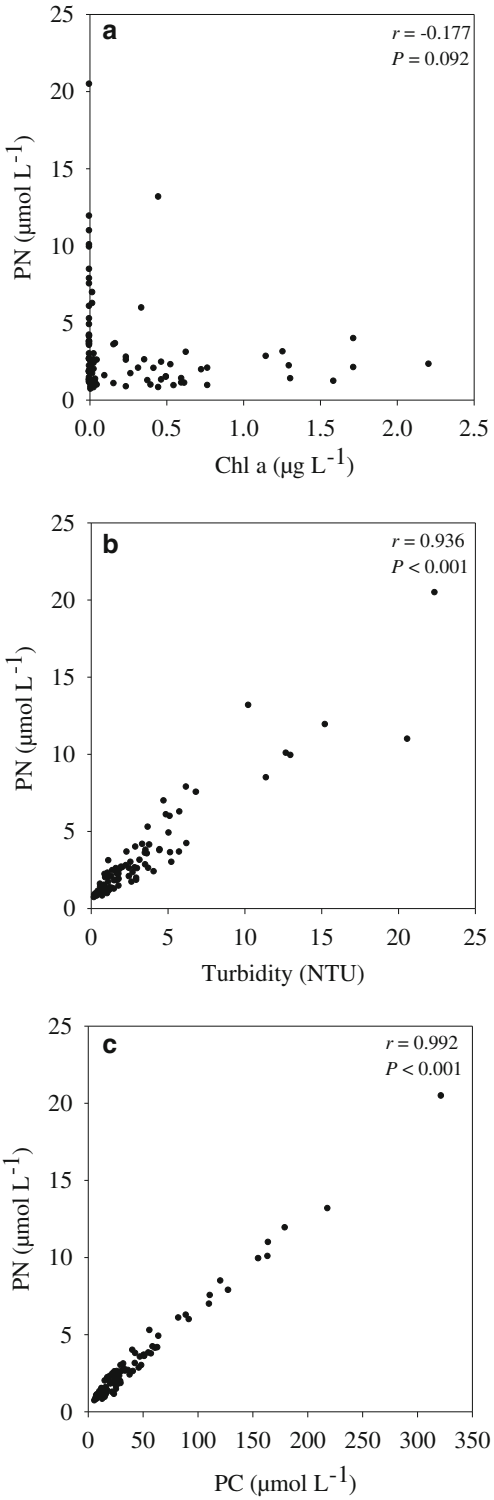

Fig. 5 Associations of (**a**) chlorophyll *a* (Chl *a*), (**b**) turbidity, and (**c**) particulate carbon (PC) with particulate nitrogen (PN) during high river flow conditions in Hilo Bay, Hawaii, USA, from 2007 to 2008. Results from correlations are shown on figure (n=92, α=0.05)

Outer Bay directly exchanges with the ocean, whose dominant form of N in the surface waters is DON (reviewed in Berman and Bronk 2003), whereas the Wailoa River plume is greatly affected by groundwater draining urban and agricultural lands and whose dominant form of N is NO_3^-+ NO_2^-. N within in the Wailuku River plume was dominated by DON; however, its contribution to the N pool was intermediate between values for the Outer Bay and Wailoa River plume. Mixing of riverine and ocean water within this region can explain the pattern as ocean water dominates during low-flow conditions and Wailuku River waters dominate during storms.

DON is becoming increasingly recognized as an important form of N in estuaries as it can comprise 30 up to 80 % or more of the dissolved N (Berman and Bronk 2003; Boynton and Kemp 2008), is bioavailable to bacteria and some phytoplankton (i.e., Bronk and Glibert 1993; Carlsson et al. 1993; Seitzinger and Sanders 1997; Carlsson et al. 1999; Bronk et al. 2007), and has been implicated in the formation of some harmful coastal algal blooms (i.e., Paerl 1988; Granéli et al. 1999; Berg et al. 1997; Lomas et al. 2001; Glibert et al. 2007). However, most of our knowledge about DON's importance in estuaries is derived from temperate systems and presently little is known about tropical estuaries. A recent study in Kaneohe Bay suggests that distributions of *Synechoccus*, the dominant phytoplankton, are affected by DON concentrations (Cox et al. 2006). Findings from previous work in temperate estuaries and Kaneohe Bay highlight the need for more research on the role of DON in tropical estuaries.

Carbon

Effects of storms on the organic C pools of estuaries are less well-known than those on the N pool. In Hilo Bay, storms increased concentrations of both DOC and PC at all stations, except within the Outer Bay with regards to DOC, where it remained fairly constant between river flow conditions. Similar patterns have been observed in the temperate Neuse River estuary, North Carolina, where DOC and PC concentrations doubled following storms from low river flow values (Paerl et al. 2001). Likewise, total organic carbon (TOC) concentrations increased two to five times following hurricanes in eastern Florida Bay (Williams et al. 2008). In watersheds with significant forest cover like Hilo Bay, flushing of the litter and upper soil horizons in the riparian zone during storms has been linked to increased riverine DOC concentrations (Hornberger et al. 1994; Frank et al. 2000). In more developed watersheds like the Neuse River, increased loading of organic matter during storms has been attributed to wastewater treatment plant bypasses, leakage and runoff from animal waste facilities, cropland inundation, and flooding of underground storage tanks (Bales 2003). In Florida Bay, organic matter from the mangroves was flushed out into the estuary during storms (Williams et al. 2008). Increased PC concentrations in

estuaries during storms are most likely a result of surface runoff eroding watershed soils. Additionally, the dominant form of organic C across all stations sampled in Hilo Bay during both low- and high-flow river conditions was DOC. The same pattern was observed for the Neuse River estuary (Paerl et al. 2001). The fact that DOC is the dominant form of organic C may have important implications for the microbial food web of Hilo Bay, as DOC has been shown to be an important C source for estuarine bacteria, stimulating their production, and possibly supporting higher trophic levels (Hopkinson et al. 1998; Moran et al. 1999; Wikner et al. 1999). Results from a food web study in Hilo Bay concurrent with ours suggests that bacteria are an important link for transferring terrestrial C to higher trophic levels like micro- and mesozooplankton (Atwood et al. 2011), although this is not necessarily a universal pattern across estuaries (Sobczak et al. 2002).

Phosphorus

The most common form of P measured in estuaries following storms is PO_4^{3-} and it has been found to generally increase with flood waters, except in areas with point source discharge, where PO_4^{3-} concentrations are diluted (i.e., Eyre and Twigg 1997; Eyre and Balls 1999; Mallin et al. 2002; Peierls et al. 2003; Ringuet and Mackenzie 2005). In contrast, almost nothing is known about how storms affect DOP concentrations in estuaries. In Hilo Bay, TDP was detectable in only 22 % of the samples collected, was primarily DOP (89 %±0.05), was detected at similar levels among regions, and was not affected by storms. In Kaneohe Bay, PO_4^{3-} and DOP were not consistently affected by storms (Cox et al. 2006; De Carlo et al. 2007), and few other studies have measured the change in these two P forms following storms (McKee et al. 2000). In contrast to DOP, PO_4^{3-} concentrations differed among regions in Hilo Bay and were detected only within the Wailoa River plume. There are two possible factors contributing to the detectable PO_4^{3-} concentrations within the Wailoa River plume. First, there is a higher percentage of developed (11 %) and agricultural (4 %) lands within this watershed compared to the Wailuku River watershed (1 % developed + agriculture), and these land uses have been shown to contribute substantial amounts of PO_4^{3-} to rivers and estuaries (Bennett et al. 2001; Harrison et al. 2010). Second, PO_4^{3-} may have been released from potentially anoxic sediments within the Wailoa River plume as the bottom waters in this region had low dissolved oxygen concentrations (average: 4.52±0.21 mgO_2L^{-1}; Wiegner and Mead 2009) and PO_4^{3-} sorbed onto iron oxyhdroxides, a significant component of Hawaiian sediments (Matsusaka and Sherman 1961), is released when iron in this form is reduced to a soluble form in anoxic sediments (reviewed in Ruttenberg 2005).

Biological Parameters

Previous studies have observed washouts of estuarine plankton during storms, resulting in immediate decreases in their biomass, with the most common parameter documenting this phenomenon being Chl *a* (i.e., Flemer et al. 1977; Alpine and Cloern 1992; Eyre 2000; De Carlo et al. 2007). In Hilo Bay, Chl *a* concentrations decreased immediately following storms. High discharge from the Wailuku River is thought to have washed phytoplankton cells out of Hilo Bay, as the highest discharge measured during our study could have filled Hilo Bay's entire water column volume in less than eight days. It is also likely that any phytoplankton cells still remaining in Hilo Bay were diluted by the large amounts of debris discharging from the Wailuku River and light limited by the amount of particles suspended in the water column, as TSS and turbidity in Hilo Bay's surface waters were two and five times higher during storms, respectively (Table 2). An earlier study also suspected that

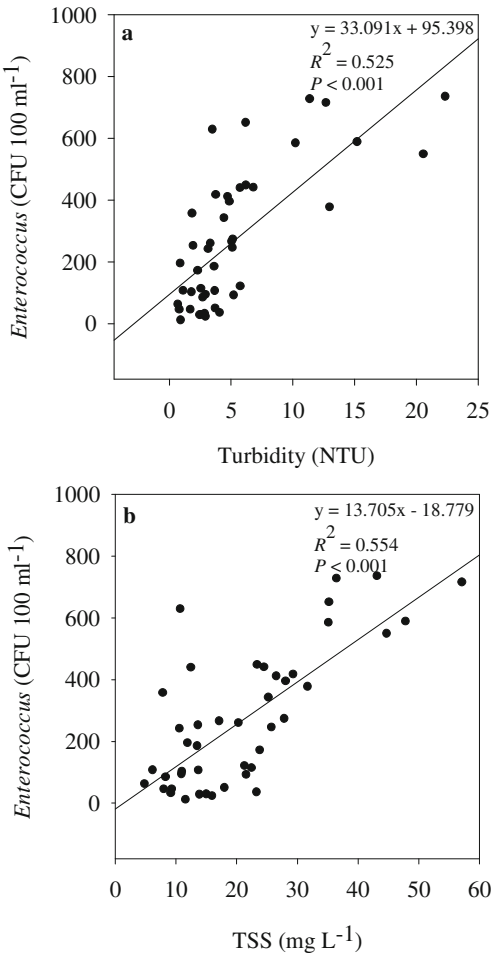

Fig. 6 Relationship between *Enterococcus* levels with (**a**) turbidity and (**b**) total suspended solids (TSS) under high river flow conditions in Hilo Bay, Hawaii, USA, from 2007 to 2008. Results from regression analyses are shown on figure (n=67, α=0.05)

salinity fluctuations in surface waters of Hilo Bay during and just after storms were too stressful for phytoplankton (M & E Pacific 1980). High zooplankton grazer abundance can also decrease Chl *a* concentrations in estuaries; however, it is unlikely that high-grazing pressure was responsible for the low Chl *a* concentrations in Hilo Bay following storms as the abundance of other types of plankton (bacteria) were lower compared to low river flow conditions.

Densities of microbial pathogens and their indicator organisms in estuarine waters are often measured following storms and have been shown to increase (Mallin et al. 2002; Burkholder et al. 2004; Mallin and Corbett 2006). In comparison, measures of total bacteria cell abundance and production in estuaries following storms are generally lacking (Cox et al. 2006; Williams et al. 2008). In Hilo Bay, total bacteria cell abundances decreased during storms. This pattern has also been documented in Kaneohe Bay and Florida Bay (Cox et al. 2006; Williams et al. 2008). A previous study in Hilo Bay found that fecal coliform bacteria and *Enterococci* abundances in surface waters were strongly correlated with rainfall and that the highest counts were near major freshwater sources (Dudley and Hallacher 1991). These two pieces of information suggest that the larger bacterial community in Hilo Bay is flushed out and/or diluted with the high river discharge during storms, but that the floodwaters introduce bacteria associated with sewage and/or soils into the bay. It is difficult to determine which of these two sources contribute *Enterococci* to Hilo Bay during storms, as these FIB have been shown to also come from soils in Hawaii and other tropical areas (Hardina and Fujioka 1991; Fujioka et al. 1999). Therefore, we analyzed for the presence of human-specific genetic markers in the *esp* gene in *E. faecium* and *E. faecalis* and found that all storm samples were negative for these markers suggesting that increases in *Enterococcus* levels during storms were from soils and not from sewage or septage inputs. Regression analysis further supports that *Enterococcus* are associated with soils during storms as they are strongly predicted by turbidity and TSS (Fig. 6). *Enterococcus* levels during low-flow conditions were below the sensitivity detection levels for *esp* genetic marker detection and so fecal pollution indicator sources could not be determined during these conditions.

Implications

With the increased number and intensity of storms predicted for the Pacific and Atlantic basins with global warming (Emanuel 2005; Webster 2005), estuarine water quality in many regions of the world will become more greatly affected by storms. From these climate predictions, it can be extrapolated that estuaries will be more frequently impacted by storms and that storm effects on water quality will be greater and longer lasting due to increased storm intensity. This may be especially true for the windward side of Hawaii Island, where Hilo Bay is located, and also along the equatorial Pacific and Atlantic as climate models predict increased precipitation and storms for these regions with increased global warming (Chu et al. 2010; Xie et al. 2010). Additionally, work from the Cape Fear River estuary, North Carolina, documented that water quality degradation by storms was considerably increased by human activities in the watershed (Mallin et al. 1999). Therefore, impacts of future storms may be even more devastating to estuarine water quality than anticipated as more human-derived pollutants will be discharged to coastal waters, especially in areas with increasing populations and development like the tropics. Our research in Hilo Bay demonstrates that storms and watershed land use can affect tropical coastal water quality and it highlights the need for more research on the effects of these two factors, as well as their interaction, on tropical estuarine water quality as more storms and greater development are predicted for the tropics.

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