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Marine Pollution Bulletin xxx (2012) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Algal $\delta^{15}N$ values detect a wastewater effluent plume in nearshore and offshore surface waters and three-dimensionally model the plume across a coral reef on Maui, Hawai'i, USA

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ARTICLE INFO

Keywords:
$\delta^{15}N$
Wastewater
Nitrogen
Algal bioassay
Coral reefs

ABSTRACT

The coral reef at Kahekili, Maui is located ~300 m south of the Lahaina Wastewater Reclamation Facility which uses four Class V injection wells to dispose of 3–5 million gallons of wastewater effluent daily. Prior research documented that the wastewater effluent percolates into the nearshore region of Kahekili. To determine if the wastewater effluent was detectable in the surface waters offshore, we used algal bioassays from the nearshore region to 100 m offshore and throughout the water column from the surface to the benthos. These algal bioassays documented that significantly more wastewater effluent was detected in the surface rather than the benthic waters and allowed us to generate a three-dimensional model of the wastewater plume in the Kahekili coastal region. Samples located over freshwater seeps had the highest $\delta^{15}N$ values (~30–35‰) and the effluent was detected in surface samples 500 m south and 100 m offshore of the freshwater seeps (~8–11‰).

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1. Introduction

Anthropogenic nutrient loading of coastal waters has had major impacts on the receiving ecosystems worldwide. For example, in response to heavy nitrogen (N) loads large scale blooms of opportunistic macroalgae have formed in tropical and temperate regions in coral reef and estuarine systems (Lapointe, 1997; Paerl, 1997; Valiela et al., 1997; McCook, 1999; Stimson et al., 2001; Lapointe et al., 2005; Morand and Merceron, 2005; Viaroli et al., 2005; Pinon-Gimate et al., 2009). Algal blooms have various negative affects on ecosystems, including reduced oxygen levels via the decomposition of algal tissue, increased microbial abundance, and emigration of fish from the impacted area (Rosenberg, 1985; Alber and Valiela, 1994; Morand and Briand, 1996; Lapointe, 1997). Specifically, on coral reefs in tropical regions, N-driven algal blooms growing over corals can affect their nutrition, growth and survival by smothering and limiting light levels (Smith et al., 1981). Coral mortality has also resulted from high phosphate concentrations from sewage-derived wastewater effluent (wastewater effluent) through the inhibition of calcification and localized bacterial infection (Kinsey and Davies, 1979; Walker and Ormond, 1982).

Nutrient sources of fertilizer and sewage can be differentiated through the use of stable isotopes of N (^{15}N:^{14}N, expressed as $\delta^{15}N$; Eq. (1)) because natural (atmospheric) and fertilizer N sources have generally low values ranging from 0‰ to 4‰ and −4 to 4‰, respectively (Owens, 1987; Macko and Ostrom, 1994) and sewage N sources are enriched in ^{15}N ranging from 7‰ to 38‰ (Kendall, 1998; Gartner et al., 2002; Savage and Elmgren, 2004). The elevated $\delta^{15}N$ values found in sewage N arises from the denitrification of nitrate and nitrification of ammonia during which fractionation occurs by microbes for the easier to metabolize, lighter isotope (^{14}N) (Heaton, 1986). The volatilization of ^{14}N-ammonia also enriches the sewage N source in ^{15}N relative to ^{14}N (Heaton, 1986). The release of N_2 into the atmosphere from the natural, microbe driven processes of nitrification and denitrification (Biological Nitrogen Removal (BNR)) is now used by some wastewater treatment facilities to reduce N levels in the effluent (Wiesmann, 1994; Zumft, 1997). Therefore, the wastewater effluent from facilities using BNR for N removal will likely have highly elevated $\delta^{15}N$ values.

Because algae assimilate N from their surrounding environment into their tissues, algal $\delta^{15}N$ values have been used worldwide to discriminate between anthropogenic and natural N sources and map the presence of anthropogenic N in a variety of coastal environments (Lapointe, 1997; Riera et al., 2000; Gartner et al., 2002; Umezawa et al., 2002; Savage and Elmgren, 2004; Steffy and Kilham, 2004; Barlie and Lapointe, 2005; Deutsch and Voss, 2006; Lin et al., 2007; Thornber et al., 2008; Pitt et al., 2009; Dailer

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doi:10.1016/j.marpolbul.2011.12.004

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et al., 2010). The $\delta^{15}N$ values of algae growing adjacent to wastewater outfalls have ranged from 8‰ to 19‰ (Costanzo et al., 2001; Gartner et al., 2002; Barlie and Lapointe, 2005; Lin et al., 2007; Thornber et al., 2008; Pitt et al., 2009). To date, the highest reported algal $\delta^{15}N$ value is 50.1‰ from samples deployed over warm freshwater seeps on a coral reef suspected to be affected by wastewater effluent at Kahekili on the island of Maui (Dailer et al., 2010). In agreement with other studies (Costanzo et al., 2001, 2005) Dailer et al. (2010), verified that the $\delta^{15}N$ values of transplanted algae express the integration of new N sources over short time intervals of less than one week and can be used to map the presence of wastewater effluent. Thus, considering the findings from Dailer et al. (2010), and the likelihood that non-saline wastewater effluent would rise to the surface waters as a buoyant plume, this study aimed to use algal bioassays to (1) determine if there is a difference in the presence of the wastewater effluent in the surface versus the benthic waters and (2) map the wastewater effluent plume as a three-dimensional model, across the coral reef at Kahekili.

2. Study area

The study area is within the Kahekili Herbivore Fisheries Management Area (HFMA) that was established to restore a healthy grazing population in July 2009 by the State of Hawai'i, Department of Land and Natural Resources, Division of Aquatic Resources (http://Hawaii.gov/dlnr/dar/regulated_areas_maui.html). The Kahekili HFMA spans approximately 1.5 km of coastline and is closed to the taking of herbivorous fishes and sea urchins in an attempt to battle prolific algal growth associated with the area's decline in coral cover from 55% to 33% over the past decade (http://Hawaii.gov/dlnr/dar/pubs/MauireefDeclines.pdf). The Lahaina Wastewater Reclamation Facility (WWRF) is located about 300 m north of the study area approximately 900 m from the coastline. This facility uses BNR to reduce N concentrations in the wastewater effluent (Parabocoli, personnel communication) and operates four Class V injection wells for wastewater effluent disposal. An injection well is a bored, drilled or driven shaft, whose depth is greater than its largest surface dimension; or a subsurface fluid distribution system used to discharge fluids underground (Code of Federal Regulations, Chapter 40 Part 144.3). The shallow forereef at Kahekili (~1.5–10 m offshore) has had algal blooms (primarily of *Ulva fasciata*) in the summer months when the wave action is negligible in the area (MD pers. obs.). The shallow forereef also has warm, continuously flowing freshwater seeps that are surrounded by rocks and coral rubble with black precipitates. Although these black, powdery and impregnating minerals have not been chemically analyzed yet, we believe that they are likely manganese oxides and/or iron oxyhydroxides which oxidatively precipitate from the solution exiting the seeps upon encountering normally oxygenated benthic waters (Glenn, personnel communication; Roden et al., 2004; Konhauser, 2007). The most persistent current in the area flows from the north to the south (Storlazzi and Field, 2008).

3. Material and methods

3.1. Three-dimensional algal bioassay deployments

In our previous study, we aimed to determine the two-dimensional extent of the Lahaina WWRF wastewater effluent plume across the coral reef at Kahekili and used nearshore and offshore sites (32 total) for algal bioassay deployments of *U. fasciata* 0.5 m from the benthos (Dailer et al., 2010). Those experiments revealed that the nearshore sites in the south were still located within the wastewater effluent plume and that the offshore sites (at 6.0 m depth) probably underestimated the plume boundaries because the non-saline wastewater effluent is likely more buoyant than the surrounding saltwater. Building on our previous field experiments, we expanded the experimental area by adding one transect in the north, two transects in the south and extending the array to the surface (at sites deeper than 1.5 m) with algal samples stratified throughout the water column (Fig. 1). The expanded array consisted of 45 sites total. Nine transects extended offshore each with four sites A–D at the following depths (m): A ~1.5, B ~2.0, C ~3.0 and D ~6.0 (Fig. 1); and an additional nine sites (S1–S8 and NS) were located in the shallow (~1.5 m depth) nearshore area containing the warm freshwater seeps (Fig. 2). Two sites, B4 and NS, were located directly over warm, freshwater seeps.

Samples of *U. fasciata* were collected from a bloom location at Waipuilani Beach Park (with initial $\delta^{15}N$ values ~5‰) and acclimated to low nutrient seawater for seven days to deplete internal N stores. After acclimation, samples were placed in 10 × 10 cm cages enclosed in plastic mesh and attached to float lines at the specified locations ($n = 3$ per site, per depth) (Figs. 1 and 2). The perimeter of the array consisted of large (0.5 m × 0.5 m) Aqua Lantern bouys equipped with solar panels to charge four AA batteries during the day that would automatically turn on five internal LED bulbs at night to provide a lit perimeter for boaters. The array was deployed for seven days in February, April, May and June 2010. In February, the array consisted of 261 samples and the results from the most northern and southern transects indicated that the wastewater effluent was still strongly present at these locations; we therefore, further expanded the array by adding one transect in the north and shifting the two most southern transects farther south. The algal bioassay deployments for April, May and June consisted of 291 samples and spanned 900 m of the Kahekili HFMA. A total of 1098 samples were deployed across all deployment periods, of which 951 samples were recovered (a recovery rate of ~87%). Some samples were not recovered because they were disintegrated in large unexpected wave events.

3.2. Algal sample preparation

Field and acclimated samples were prepared in triplicate to obtain the initial and acclimated $\delta^{15}N$ values. Recovered samples from each deployment (per site, per depth) were prepared for final $\delta^{15}N$ values. Samples were rinsed in deionized water, dried at 60 °C to a constant weight, and ground with mortar and pestle into a powder. Powdered samples were then sent for tissue $\delta^{15}N$ determinations to the Biogeochemical Stable Isotope Laboratory,

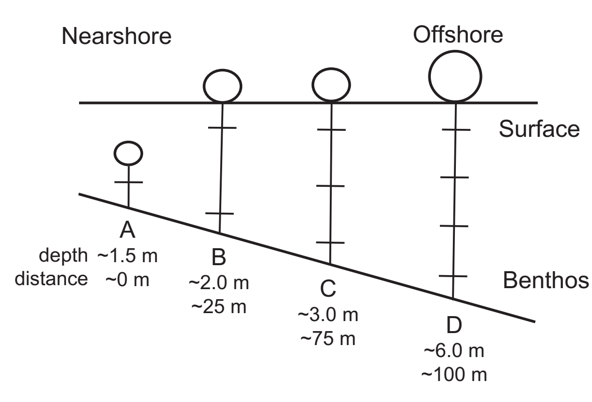

Fig. 1. Schematic of one transect for the algal bioassay deployments extended from the benthos to the surface. Sites A–D are shown from nearshore to offshore with accompanying approximate depths (m) and distances (m) from shore. Each horizontal dash represents a cluster of three mesh cages with one sample of acclimated *Ulva fasciata* per cage.

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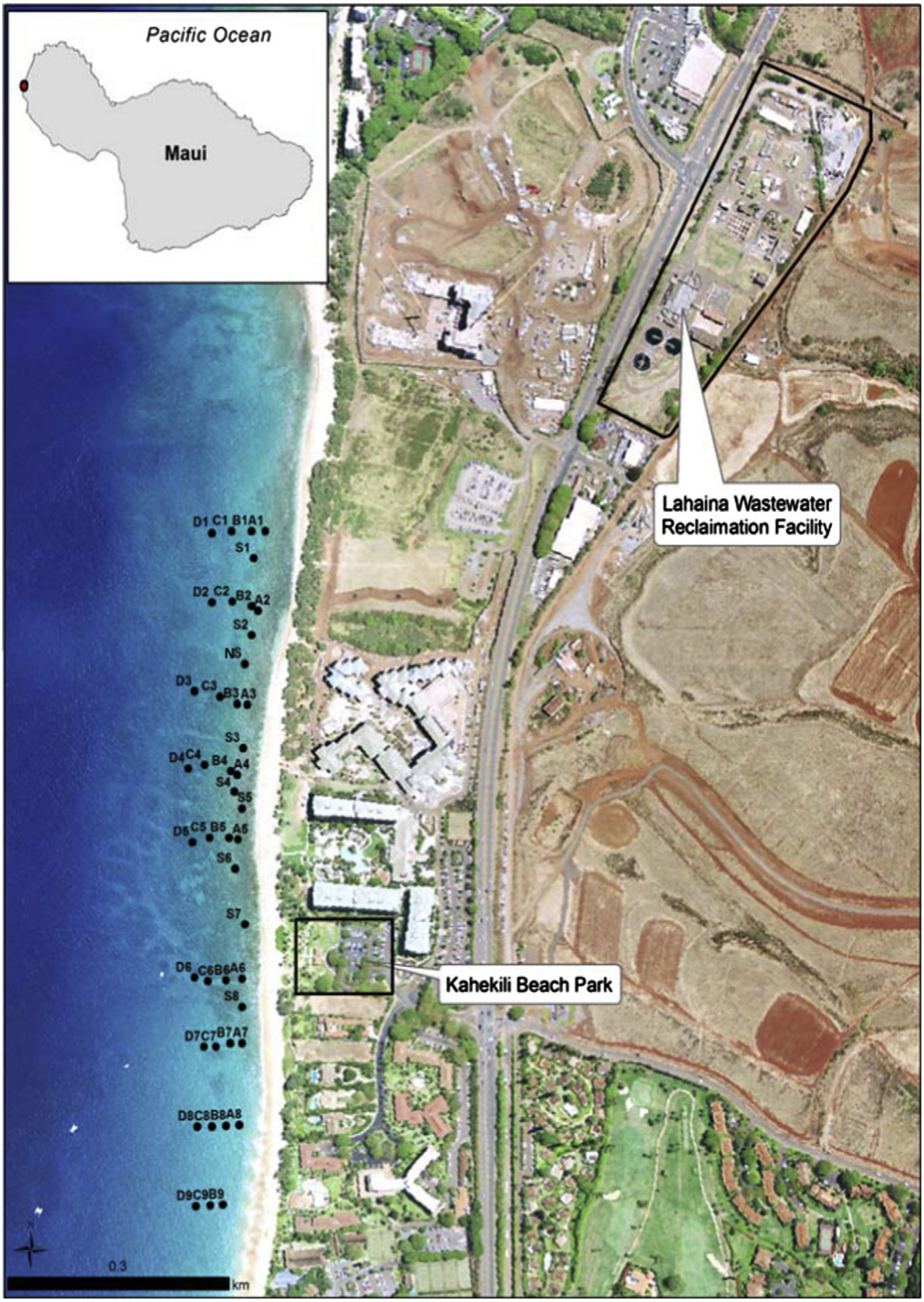

Fig. 2. Algal bioassay deployment sites across the coral reef at Kahekili. Aerial imagery was acquired through the Hawai'i Coastal Geology Group, School of Ocean and Earth Sciences and Technology (2007). <http://www.soest.hawaii.edu/coasts/erosion/maui/aerials.php>.

University of Hawai'i at Manoa. Samples were weighed then analyzed with a Carlo Erba NC 2500 Elemental Analyzer, Finnigan MAT ConFloII, and Finnigan MAT DeltaS. Ratios of ^{15}N:^{14}N were expressed relative to atmospheric nitrogen and calculated as (Sweeney et al., 1978):

$$\delta^{15}\mathrm{N}\ (‰) = \{(R_{\mathrm{sample}}/R_{\mathrm{standard}}) - 1\} \times 10^3,\ \text{where } R = {}^{15}\mathrm{N}/{}^{14}\mathrm{N} \quad (1)$$

3.3. Statistical analyses

Data from the algal bioassays were not normally distributed and had unequal sample sizes per site, per depth by the end of the deployments due to unexpected large wave events that disintegrated some of the samples; therefore the data were analyzed with a General Linear Model with Type III error. After a significant result (see below for results by deployment for surface and benthic

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locations) an Unequal N post hoc test was performed to determine significance of algal $\delta^{15}N$ values within deployment sites and initial levels (field and acclimated samples) (performed with Satistica 6.0). Sites with only one remaining sample were not included in statistical analyses. Simple linear regressions were used to determine if there were significant relationships in the $\delta^{15}N$ values from the surface to the benthos per offshore location (B, C and D) per deployment (performed with SigmaPlot 9.0).

3.4. Three-dimensional modeling of the Lahaina WWRF wastewater effluent plume

The Lahaina WWRF wastewater effluent plumes for February, April, May and June 2010 were created with EnviroInsite, which is a three-dimensional groundwater visualization software. A Garmin GPS 76CS Plus was used to obtain the GPS coordinates in WGS 84 for algal bioassay deployment sites. The wastewater effluent plumes were modeled using an Inverse Distance Weighting algorithm with the following parameters: Z scale = 0.5 (>1 for anisotropy), exponent (n) = 1.5, smooth distance = 1, and search radius = 850 m (the extent of all sites). The modeled wastewater effluent plumes cover an area of approximately 80,275 m^2 with resolution or cell size of approximately 2.0 m $\times$ 17.0 m.

4. Results

A significant effect of site was found for each deployment month for the surface and benthos (GLM ANOVA): February surface, $F_{39,74} = 44.7$, $P < 0.00001$; February benthos, $F_{39,67} = 59.0$, $P < 0.00001$; April surface, $F_{42,64} = 27.8$, $P < 0.00001$; April benthos, $F_{40,58} = 44.1$, $P < 0.00001$; May surface, $F_{42,72} = 40.8$, $P < 0.00001$; May benthos, $F_{42,71} = 87.7$, $P < 0.00001$; June surface, $F_{43,68} = 17.4$, $P < 0.00001$; June benthos, $F_{43,65} = 58.5$, $P < 0.00001$. Regardless of deployment month, all samples deployed over warm freshwater seeps drastically and significantly ($P < 0.0002$) increased in $\delta^{15}N$ values from ~5‰ initially to 30.7 ± 1.1‰ in February, 35.4 ± 0.5‰ in April, 31.0 ± 1.2‰ in May and 32.7 ± 0.1‰ in June (Fig. 3). For all deployments, significantly ($P < 0.02$) increased algal $\delta^{15}N$ values were observed throughout the shallow nearshore region (sites A, B and S1–S8; Fig. 3). Ranges of algal $\delta^{15}N$ values for this region (excluding values from freshwater seep sites) were as follows per month: February ~22‰ to ~9.0‰, April ~29‰ to ~8.0‰, May ~24‰ to ~8.0‰, and June ~20‰ to ~8.0‰ (Fig. 3). Significantly higher than initial $\delta^{15}N$ values were continually found in samples that were deployed at the surface and benthic locations of site B9, which was ~500 m to the south of the warm freshwater seep site B4 ($P < 0.0002$, Fig. 3). In general for all deployments, the $\delta^{15}N$ values from algae deployed in the shallow sites to the south were higher than those from S1 and transect 1 to the north (Fig. 3).

For all deployments, the $\delta^{15}N$ values of samples deployed at the surface of sites C and D (~75 and ~100 m offshore, respectively) were significantly ($P < 0.05$) increased from initial values (~5‰). More specifically, this occurred at the following sites with the accompanying range of algal $\delta^{15}N$ values per deployment: February C3–9 (~19‰ to ~10‰) and D3–9 (~12‰ to ~9‰); April C3, C6–8 (~15‰ to ~12‰) and D8–9 (~12‰ to ~11‰); May C2–3, C5–9 (~17.0‰ to ~11‰) and D3, D5, D7–9 (~11‰ to ~9‰); and June C2–7, C9 (~17‰ to ~11‰) and D2 (~11‰) (Fig. 3). However, the $\delta^{15}N$ values of samples deployed at the benthos of C and D locations generally increased but were not significantly elevated from initial values (Fig. 3). The $\delta^{15}N$ values of algae deployed at B sites located ~25 m offshore were significantly ($P < 0.04$) elevated from initial values for the following months in the surface and the benthos, respectively: April B4, B6–9 and B8–9; May B2–9 and B3–5, B9; and June B2–4, B6–9 and B2–5, B9 (Fig. 3). In February, all B sites were significantly ($P < 0.03$) elevated from initial values in both the surface and the benthos (Fig. 3). The three-dimensional models of the wastewater effluent plumes show that the highest $\delta^{15}N$ values were located in the nearshore warm freshwater seep zone and that the effluent was detected on the most southern transect, regardless of deployment month (Fig. 4).

For each deployment, a significant ($P < 0.0009$) decreasing relationship was found in the $\delta^{15}N$ values of algae deployed at the surface through the water column to the benthos for all of the offshore locations (B, C and D) except for site B4 (Table 1). B4 was the only offshore site located directly over a warm freshwater seep. The pooled data from site B4 across deployment months for the surface and benthos, separately, showed a significant ($P < 0.0008$) increasing relationship from the surface to the benthos (Table 1). This shows that regardless of deployment month, the wastewater effluent was more detectable in the surface than the benthos waters at all offshore sites except for B4 where a warm freshwater seep was located. In summary, these data demonstrate that the Lahaina WWRF effluent plume (1) affected the majority of the shallow region at Kahekili, (2) rose to the surface waters in the area and (3) generally flowed south with the most predominant current in the area (Fig. 3 and 4).

5. Discussion

Algae with high N uptake rates generally quickly respond to pulses of nutrients (Wallentinus, 1984) and can be used as an additional method to assess water quality in coastal environments. Algal bioassays can be deployed in an area of concern to integrate water column N over short time periods to examine dominant source(s) of N in the area (Costanzo et al., 2001, 2005). Although no macroalgal specific evidence of isotopic preference (fractionation) exists (Cohen and Fong, 2005), it is possible that algal $\delta^{15}N$ values may be lowered in N rich environments (Pennock et al., 1996). However, algal $\delta^{15}N$ values have been used globally to detect sources of anthropogenic N in coastal systems and in all cases where algal tissue has been used to trace wastewater effluent, the $\delta^{15}N$ values nearest the outfalls or treatment facilities were elevated relative to natural signatures (Risk et al., 2009 and references therein). High wastewater loadings have been associated with elevated $\delta^{15}N$ values in harmful algal blooms where the wastewater effluent discharged through injection wells quickly rose to the surface likely affecting coral reefs in the area (Lapointe et al., 2005).

Dailer et al. (2010) detected the presence of the Lahaina WWRF wastewater effluent in the nearshore area of Kahekili through elevated $\delta^{15}N$ values in attached intertidal algae and algal bioassays. Since the non-saline, wastewater effluent plume is likely more buoyant than saltwater, this study extended the algal bioassay array to the surface waters at sites 25–100 m offshore. The extension to the surface waters was necessary to determine if the wastewater plume was detectable in the offshore surface waters while remaining undetected in the benthic waters (~6.0 m depth) until water column mixing occurs from large wave events.

All samples placed over warm freshwater seeps drastically and significantly increased in $\delta^{15}N$ value (from ~5‰ to ~30‰ to 35‰), regardless of deployment month. These $\delta^{15}N$ values are higher than those reported from the Lahaina WWRF (~20‰) (Hunt and Rosa, 2009). The increased difference in $\delta^{15}N$ values might be caused by continual denitrification processes in the wastewater effluent as it flows to the ocean and/or variable $\delta^{15}N$ values of the effluent. $\delta^{15}N$ values from ~30‰ to 35‰ are comparable to values reported from a wastewater treatment facility using BNR (38.0‰ Savage and Elmgren, 2004) and are higher than those reported of algae with anthropogenic exposure (25.7‰ Riera et al., 2000; 19.6‰ Jones et al., 2001).

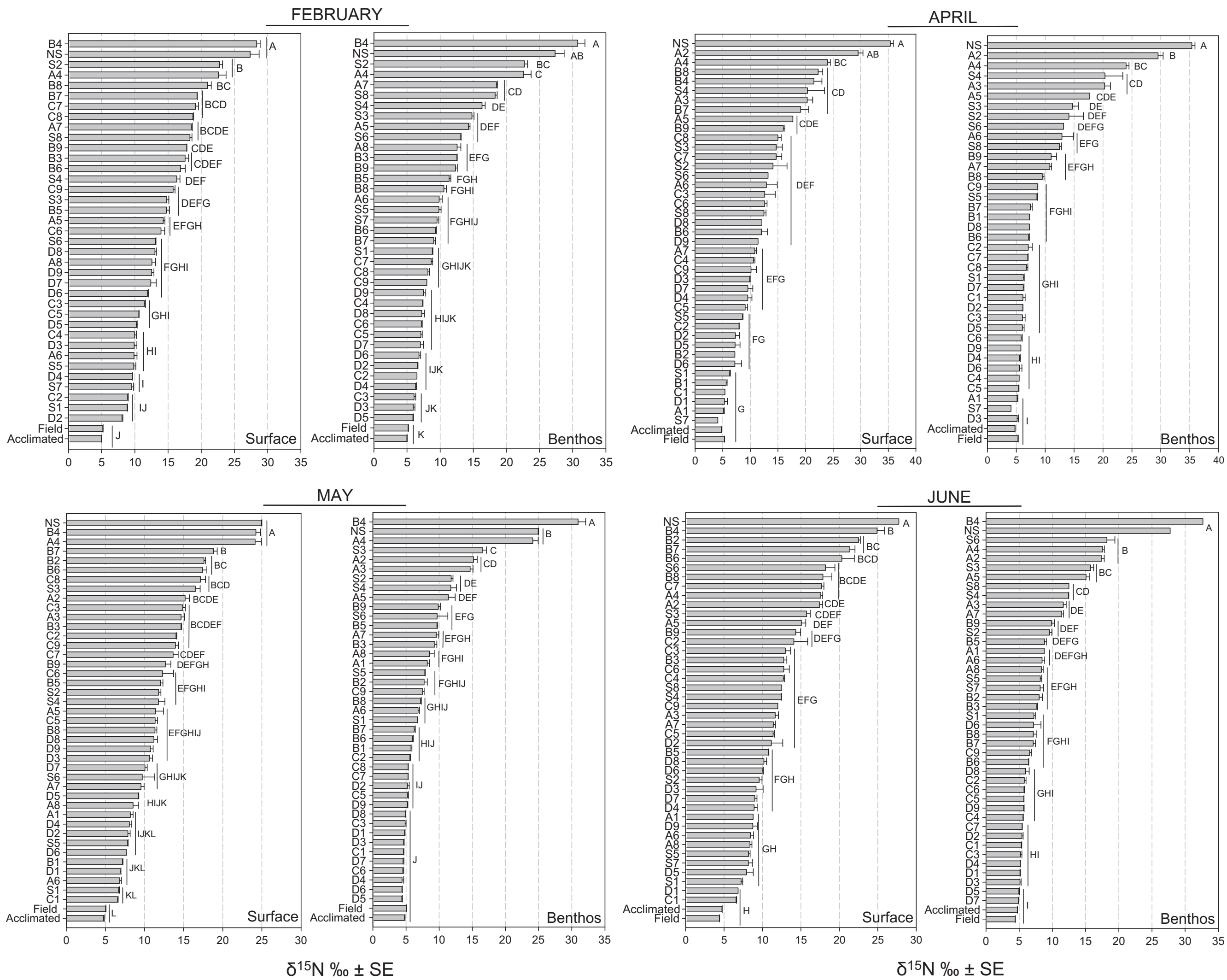

Fig. 3. February, April, May and June 2010 $\delta^{15}N$ values (average ± SE) of *Ulva fasciata* for field, acclimated, and deployed samples at Kahekili at the respective sites for surface and benthic locations. Significant differences are represented by different letters; note the change in scale on the *x*-axis.

In general for all deployment months, $\delta^{15}N$ values of algae deployed at the shallow southern sites were higher than those from the most northern sites. This study agrees with the consideration that the promontory landmass of Honokowai Point likely diverts the wastewater effluent plume to the south over the coral reef at Kahekili (Dailer et al., 2010). In addition, increased $\delta^{15}N$ values to the south are consistent with the findings of Dailer et al. (2010) and research conducted by the US Geological Survey which documented that the most dominant nearshore current in the area flows from north to south (Storlazzi and Field, 2008). The $\delta^{15}N$ values of surface samples at sites ~75 m and ~100 m offshore were significantly increased from initial values (ranging from 9‰ to 19‰ across all deployments), whereas those of the benthic samples were elevated but not significantly increased. This study shows that the surface array is a successful method for the detection of wastewater effluent and that, for the Kahekili area, the wastewater signal is stronger in the surface waters than in the benthic waters when the water column is stratified from calm conditions. During large wave events however, the water column becomes well mixed and the presence of the effluent can be strongly detected at the benthic locations of the offshore sites (Dailer et al., 2010).

These results confirm that the wastewater effluent flows through the coral reef at Kahekili into the surface waters, where most of the recreational users (swimmers, snorkelers, canoe paddlers, etc.) are active, and then flows to the south. The confirmation of the wastewater effluent encompassing the nearshore and the majority of the surface waters at Kahekili potentially threatens the health of those using the ocean and marine life (e.g. fish, sea turtles, and marine mammals) in this area. This is because wastewater effluent will normally contain an assortment of microbial (bacterial and viral) assemblages (Tree et al., 2003) prior to disinfection. Radiation from ultraviolet light (UV, 254 nm) disinfects wastewater effluent by killing more than 99% of the coliform, fecal coliform, fecal streptococci and heterotrophic bacteria found in wastewater (Oliver and Cosgrove, 1975). The Lahaina WWRF is currently capable of disinfecting about 1.0 million gallons of wastewater effluent per day with UV radiation and processes an average of ~3.4 million gallons of wastewater effluent per day with an accompanying mass load estimate of 79–97 kg (174–215 lbs) per day of total nitrogen (Dailer et al., 2010). The remaining ~2.4 million gallons of wastewater per day is directed into the injection wells prior to full disinfection. Therefore, to protect the health of the recreational users and marine life of this area from

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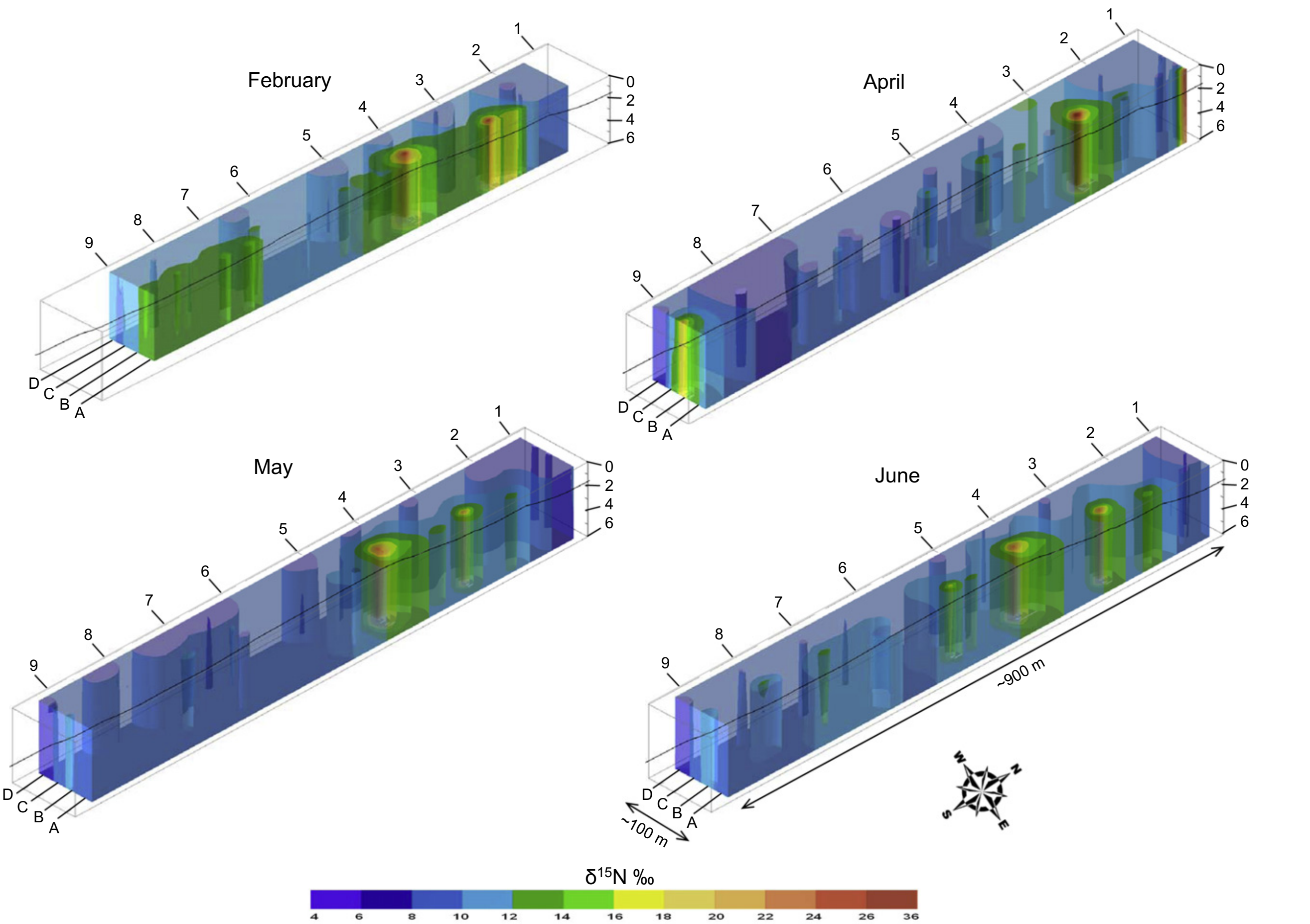

Fig. 4. Three-dimensional models of the wastewater effluent plume spanning the coral reef at Kahekili. The models were generated with EnviroInsite software from the $\delta^{15}N$ values of deployed algae in February, April, May and June 2010. Algal bioassay transects 1 through 9 are shown from north to south with deployment locations A–D from nearshore to offshore and the depth is shown from the surface (0 m) to the benthos (6 m).

Table 1
Relationships between $\delta^{15}N$ values of deployed algal samples from the surface through the benthos for all offshore sites per deployment month. For site B4, data were pooled across deployments for the surface and benthos, separately.

	Site	Equation	F	r^2	P
February	B	$y = -6.83x + 24.7$	102.6	0.76	<0.0001
	C	$y = -3.11x + 16.0$	61.4	0.48	<0.0001
	D	$y = -1.39x + 11.6$	98.5	0.52	<0.0001
April	B	$y = -7.17x + 23.0$	13.8	0.32	0.0009
	C	$y = -2.27x + 12.7$	46.4	0.41	<0.0001
	D	$y = -0.91x + 8.97$	41.7	0.33	<0.0001
May	B	$y = -2.88x + 14.5$	46.2	0.52	<0.0001
	C	$y = -1.64x + 11.9$	92.8	0.59	<0.0001
	D	$y = -0.74x + 8.45$	174.3	0.63	<0.0001
June	B	$y = -4.23x + 17.9$	58.9	0.63	<0.0001
	C	$y = -1.60x + 12.0$	75.0	0.56	<0.0001
	D	$y = -0.60x + 8.68$	53.8	0.44	<0.0001
All months freshwater seep site B4		$y = 6.72x + 17.7$	17.7	0.54	0.0008

the potential microbial assemblages associated with the wastewater effluent, the Lahaina WWRF should have the capacity to disinfect the total volume of effluent processed.

In the US, injection wells are regulated under the Safe Drinking Water Act, by the Underground Injection Control (UIC) Program which manages the subsurface injection of waste fluids below, into, and above underground drinking water sources. Correspondingly, the UIC permit conditions are designed to protect underground drinking water sources (not coastal waters) from injectate pollutants. The Clean Water Act, however, prohibits the discharge of pollutants from point sources into the waters of the US without a National Pollution Discharge Elimination System permit that implements minimum wastewater treatment standards through technology-based effluent limits. We hope that this study will help guide regulatory authorities toward improving wastewater treatment standards for the Lahaina WWRF, especially full disinfection of all effluent, to consequently enhance the water quality of the Kahekili area for the benefit of the people, as well as, the environment.

Acknowledgements

This work was conducted under the Special Activities Permit #2010-39 obtained from the State of Hawai'i, Department of Land and Natural Resources, Division of Aquatic Resources. This work was funded in part by the Hawai'i Coral Reef Initiative #NA07-NOS4000193 and the State of Hawai'i, Division of Aquatic Resources, Local Action Strategy program. We thank an anonymous reviewer who provided valuable comments to improve the manuscript.

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Please cite this article in press as: Dailer, M.L., et al. Algal $\delta^{15}N$ values detect a wastewater effluent plume in nearshore and offshore surface waters and three-dimensionally model the plume across a coral reef on Maui, Hawai'i, USA. Mar. Pollut. Bull. (2012), doi:10.1016/j.marpolbul.2011.12.004