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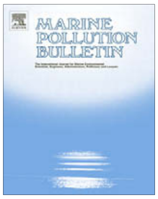

Using δ^{15}N values in algal tissue to map locations and potential sources of anthropogenic nutrient inputs on the island of Maui, Hawai‘i, USA

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ABSTRACT

Macroalgal blooms of *Hypnea musciformis* and *Ulva fasciata* in coastal waters of Maui only occur in areas of substantial anthropogenic nutrient input, sources of which include wastewater effluent via injection wells, leaking cesspools and agricultural fertilizers. Algal δ^{15}N signatures were used to map anthropogenic nitrogen through coastal surveys (island-wide and fine-scale) and algal deployments along nearshore and offshore gradients. Algal δ^{15}N values of 9.8‰ and 2.0–3.5‰ in Waiehu and across the north-central coast, respectively, suggest that cesspool and agricultural nitrogen reached the respective adjacent coastlines. Effluent was detected in areas proximal to the Wastewater Reclamation Facilities (WWRF) operating Class V injection wells in Lahaina, Kihei and Kahului through elevated algal δ^{15}N values (17.8–50.1‰). From 1997 to 2008, the three WWRFs injected an estimated total volume of 193 million cubic meters (51 billion gallons) of effluent with a nitrogen mass of 1.74 million kilograms (3.84 million pounds).

1. Introduction

Anthropogenic nitrogen (N) loading to the nearshore marine environment through sewage and fertilizer runoff are known to increase primary productivity in coastal systems (Doering et al., 1995; Taylor et al., 1999; Thornber et al., 2008). In extreme cases, excess nutrient loading in coastal regions has resulted in the formation and proliferation of large scale opportunistic macroalgal blooms (Brittany France, Briand, 1989; Puget Sound Washington USA, Thom and Albright, 1990; Venice Lagoon Italy, Sfriso et al., 1993; Jamaica and southeast Florida USA, Lapointe, 1997; Paerl, 1997; Valiela et al., 1997; Ebro River Delta Spain, Menendez and Comin, 2000; Ythan Estuary Scotland, Raffaelli, 2000; Kaneohe Bay Hawaii USA, Stimson et al., 2001; Lapointe et al., 2005; Morand and Merceron, 2005; Sacca di Goro Italy, Viaroli et al., 2005; southeastern Gulf of California USA, Pinon-Gimate et al., 2009). Ecosystem impacts of large scale algal blooms include diminished water column oxygen levels, negative effects on seagrass beds, fisheries and benthic community composition and increased microbial abundance (Barnes, 1973; Johannes, 1975; Smith et al., 1981; Rosenberg, 1985; Burkholder et al., 1992; Zaitsev, 1992; Alber and Valiela, 1994; Morand and Briand, 1996; McCook, 1999; Raffaelli, 2000).

Sources of additional N entering the ocean are often difficult to detect with many water quality assessment tools (ambient nutrient and salinity measurements) because the ocean is a dynamic environment where currents, wave activity and general mixing events can rapidly dilute potentially elevated nutrient levels. Additionally biological uptake of nutrients may occur at rates similar to input rates making the detection of nutrient flux extremely difficult. The United States Environmental Protection Agency (US EPA) recommends the use of bioassays, biological and habitat data in addition to chemical data for water quality assessments (US EPA, 2002). The use of natural stable isotopes of N (^{15}N:^{14}N, expressed as δ^{15}N) to distinguish between natural and sewage derived N is well established (see Risk et al., 2009 for a recent review) because natural (atmospheric) and fertilizer N sources have generally low signatures (ranging from 0–4 and −4 to 4‰, respectively, (Owens, 1987; Macko and Ostrom, 1994)). Sewage N is enriched in ^{15}N because bacteria preferentially use ^{14}N (Heaton, 1986) thereby elevating sewage derived wastewater in ^{15}N relative to ^{14}N. The extent of ^{15}N enrichment in sewage is therefore dependant upon on the level and type of treatment (i.e. the greater the denitrification via bacterial activity the higher the δ^{15}N value). Consequently, sewage derived δ^{15}N values in the literature from various sources of sewage range from 7‰ to 38‰ (Kendall, 1998; Gartner et al., 2002; Savage and Elmgren, 2004; summarized in Table 1).

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Table 1
Macroalgal and source (when provided) δ^{15}N values from across the world in natural and anthropogenic nitrogen loading areas. Sources of anthropogenic *N* include shrimp farm effluent (SFE), spetic tank effluent (STE), percolation ponds (PP), wastewater (WW), sewage effluent (SE), wastewater treatment plant sewage effluent (WWTP SE), secondarily treated sewage effluent (2nd WWTP SE) and effluent treated with Biological Nitrogen Removal (BNR WWTP SE).

Algal species	δ^{15}N algae	δ^{15}N source	N source	Habbitat	Location	Country	References
		0.0	Atmospheric Nitrogen	Natural			Owens (1987)
		0.0–3.0	Inorganic fertilizer	Agriculture			Owens (1987)
Cladophora sericea	0.2		Natural or Agriculture	Brackish stream	Honokohau Stream, Northwest Maui	USA	This study
Multiple genera	0.01–1.4		Natural	Lava flow	Keanae Point to Wainapanapa, Northeast Maui	USA	This study
Multiple genera	1.2–2.0		Natural	Offshore reef	Negril Marine Park	Jamaica	Lapointe and Thacker (2002)
		1.3–3.7	Natural	Coastal	Narragansett Bay, Rhode Island	USA	Thornber et al. (2008), Chaves (2004)
Multiple genera	1.3–3.0		Agriculture	Sandy beach	Spreckelsville, North-central Maui	USA	This study
Acanthophora spicifera	1.4		Natural	Estuarine	Southwestern coast of Puerto Rico	USA territory	France et al. (1998)
Gracilaria vermiculophylla	1.4–5.4	2.1	Agriculture	Coastal lagoon	Southeastern Gulf of California	Mexico	Pinon-Gimate et al. (2009)
Asteronema breviarticulatum	1.5–2.0		Natural	Basalt and Sandy beach	Makahuna Gulch, South-central Maui	USA	This study
Multiple genera	1.8		Natural	Offshore reef	Golden Grotto, Green Turtle Cay	Commonwealth of the Bahamas	Barlie and Lapointe (2005)
Ulva fasciata	1.9		Agriculture	Sandy beach	Sugar Beach, South-central Maui	USA	This study
Ulva lactuca	1.9–3.9		Natural	Nearshore reef	Green Island	Taiwan	Lin et al. (2007)
Multiple genera	1.9–3.8		Natural	Lava flow	La Perouse, Southeast Maui	USA	This study
Hypnea musciformis	1.9–3.6		Agriculture	Sandy beach	Tavares Bay, North-central Maui	USA	This study
Catenella nipae	2.0		Natural	Offshore Island	Moreton Bay	Australia	Costanzo et al. (2004)
Dictyota	2.0		Natural	Offshore reef	Ishigaki Island	Japan	Umezawa et al. (2002)
Padina	2.0		Natural	Offshore reef	Ishigaki Island	Japan	Umezawa et al. (2002)
Enteromorpha intestinalis	2.3		Natural	Nearshore reef	Green Island	Taiwan	Lin et al. (2007)
Hypnea musciformis	2.8–4.1		Agriculture	Sandy beach	Hookipa Beach Park, North-central Maui	USA	This study
Catenella nipae	2.9		Natural	Offshore island	Moreton Bay	Australia	Jones et al. (2001)
Catenella nipae	<3.0		Natural	Offshore island	Moreton Bay	Australia	Costanzo et al. (2001)
Laurencia intricata	<3.0		Natural	Offshore reef	South of Florida Bay	USA	Lapointe et al. (2004)
Fucus vesiculosus	3.0–4.0		Natural	Estuarine	Himmerjarden Bay, South of Stockholm	Sweeden	Savage and Elmgren (2004)
Multiple genera	3.1			Basalt	Kapalua, West Maui	USA	This study
Cladphora vagabunda	3.2–3.5		16% WW Loading	Estuarine	Sage Lot Pond Waquoit Bay, Massachusettes	USA	McClelland et al. (1997)
Hypnea musciformis	3.3–3.9		Agriculture	Sandy beach	Baldwin Beach Park, North-central Maui	USA	This study
		3.8	Natural spring water	Nearshore reef	Ishigaki Island	Japan	Umezawa et al. (2002)
Asteronema breviarticulatum	4.0		Natural	Lava flow	Big Kiawe - Arches, Southeast Maui	USA	This study
Multiple genera	4.4–4.9		SE and Agriculture	Sandy beach	Maalaea, South-central Maui	USA	This study
Catenella nipae	4.0–7.3		SFE	Estuarine	Hinchinbrook Island	Australia	Costanzo et al. (2004)
Catenella nipae	4.0–11.3		WWTP SE	Estuarine	Moreton Bay	Australia	Costanzo et al. (2001)
Enteromorpha intestinalis	4.4–5.1		Natural	Nearshore reef	Dakwan	Taiwan	Lin et al. (2007)
Enteromorpha intestinalis	4.6–5.2		Natural	Nearshore reef	Nanwan	Taiwan	Lin et al. (2007)
		>4.6	STE	Tequesta monitoring well #6	Southeast Florida	USA	Lapointe and Krupa (1995b)
Enteromorpha sp.	5.0		16% WW Loading	Estuarine	Sage Lot Pond Waquoit Bay, Massachusettes	USA	McClelland et al. (1997)
Multiple genera	5.0		Cesspools	Basalt	Waihikuli, West Maui	USA	This study
Acanthophora spicifera	5.3–6.0		SFE	Nearshore reef	Opunohu Bay, Moorea	French Polinesia	Lin and Fong (2008)
Cladphora vagabunda	>5.4		61% WW Loading	Estuarine	Childs River Waquoit Bay, Massachusetts	USA	McClelland et al. (1997)
Cladophra cantenata	>5.5		WWTP SE	Estuarine	South of Florida Bay	USA	Lapointe et al. (2004)
H. musciformis & U. fasciata	5.5–6.6			Basalt	Kahana, West Maui	USA	This study
Multiple genera	5.9–7.0		Cesspools	Sandy beach	Keawakapu Beach Park and Wailea, South Maui	USA	This study
Cheatomorpha linum	>6.0		WW	Nearshore reef	Negril Marine Park	Jamaica	Lapointe and Thacker (2002)
Multiple genera	6.0–12.0		SE, STE, PP	Nearshore reef	East of Central Florida	USA	Barlie (2004)
Ulva australis	6.1		Natural	Nearshore reef	Ocean Reef, Beenyup	Australia	Gartner et al. (2002)
Fucus vesiculosus	6.3		Natural	Estuarine	Oosterschelde Estuary	The Netherlands	Riera et al. (2000)
Multiple genera	6.1–6.9		Anthropogenic	Sandy beach	Makena, South Maui	USA	This study
Laurencia intricata	>6.5		WWTP SE	Estuarine	South of Florida Bay	USA	Lapointe et al. (2004)
Vidalia sp.	6.5		Natural	Nearshore reef	Ocean Reef, Beenyup	Australia	Gartner et al. (2002)

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Macroalgae	6.7		Sewered	Estuarine	Valley Creek, Pennsylvania	USA	Steffy and Kilham (2004)
H. musciformis & U. fasciata	6.8			Sandy beach	South Waipuilani Beach Park, South Maui	USA	This study
Vidalia sp.	>7.0	13.5–23.5	2nd WWTP SE	Nearshore reef	Ocean Reef, Beenyup	Australia	Gartner et al. (2002)
Catenella nipae	7.1–8.6		SFE	Estuarine	Moreton Bay	Australia	Jones et al. (2001)
Enteromorpha sp.	7.3		Natural	Estuarine	Oosterschelde Estuary	The Netherlands	Riera et al. (2000)
		>7.3	STE	Juptier Creek monitoring well #4	Southeast Florida	USA	Lapointe and Krupa (1995a)
H. musciformis & U. fasciata	6.5		WWTP	Sandy beach	West Kite Beach, North-central Maui	USA	This study
H. musciformis & U. fasciata	7.3–7.8		Anthropogenic	Sandy beach	Kaanapali, West Maui	USA	This study
Dictyota	>8.0		Anthropogenic	Nearshore reef	Ishigaki Island	Japan	Umezawa et al. (2002)
Enteromorpha intestinalis	8.0–8.6		WWTP SE	Nearshore reef	Dakwan	Taiwan	Lin et al. (2007)
Fucus vesiculosus	8.0–10.5	24.0	WWTP SE 1994–1997	Estuarine	Himmerjarden Bay, South of Stockholm	Sweeden	Savage and Elmgren (2004)
Fucus vesiculosus	8.0–13.0	38.0	BNR WWTP SE 1998–2002	Estuarine	Himmerjarden Bay, South of Stockholm	Sweeden	Savage and Elmgren (2004)
Gracilaria vermiculophylla	8.0–13.6	16.1	WWTP SE	Coastal lagoon	Southeastern Gulf of California	Mexico	Pinon-Gimate et al. (2009)
Multiple genera	>8.0		WWTP SE	Nearshore reef	Southeast Florida	USA	Lapointe et al. (2005)
Multiple genera	>8.0		SE	Estuarine	Town Harbor, Green Turtle Cay	Commonwealth of the Bahamas	Barlie and Lapointe (2005)
Padina sp.	>8.0		Anthropogenic	Nearshore reef	Ishigaki Island	Japan	Umezawa et al. (2002)
Ulva lactuca	>8.0		WWTP SE	Estuarine	South of Florida Bay	USA	Lapointe et al. (2004)
Enteromorpha sp.	8.5		61% WW Loading	Estuarine	Childs River Waquoit Bay, Massachusetts	USA	McClelland et al. (1997)
Ulva australis	8.8–12.8	13.5–23.5	2nd WWTP SE	Nearshore reef	Ocean Reef, Beenyup	Australia	Gartner et al. (2002)
Catenella nipae	9.0		WWTP SE	Estuarine	Brisban River, Moreton Bay	Australia	Costanzo et al. (2005)
Catenella nipae	9.0		WWTP SE	Estuarine	Pine River, Moreton Bay	Australia	Costanzo et al. (2005)
Vidalia sp.	9.3	13.5–23.5	Algae grown in 2nd WWTP SE	Laboratory Experiment	Ocean Reef, Beenyup	Australia	Gartner et al. (2002)
Ulva australis	9.3	13.5–23.5	Algae grown in 2nd WWTP SE	Laboratory Experiment	Ocean Reef, Beenyup	Australia	Gartner et al. (2002)
Ulva fasciata	9.8		Cesspools	Rocky beach	North Waiehu, North-central Maui	USA	This study
Enteromorpha sp.	9.9–11.9		Anthropogenic	Estuarine	Warnow System, Baltic Sea	Germany	Deutsch and Voss (2006)
H. musciformis & A. concinna	10.0		WWTP SE	Basalt wall	Kahului WWRF Resevoir, North-central Maui	USA	This study
		>11.8	STE	Tequesta monitoring well #10	Southeast Florida	USA	Lapointe and Krupa (1995b)
Flimanetous algae	12.0–15.0		BNR WWTP SE	Estuarine	Caboolture River, Moreton Bay	Australia	Pitt et al. (2009)
		12.0	Livestock farming	Effluent sample	Southeastern Gulf of California	Mexico	Pinon-Gimate et al. (2009)
Macroalgae	12.3		STE	Estuarine	Valley Creek, Pennsylvania	USA	Steffy and Kilham (2004)
Ulva sp.	12.6–13.5		Anthropogenic	Estuarine	Warnow System, Baltic Sea	Germany	Deutsch and Voss (2006)
Flimanetous algae	13.0–19.3		WWTP SE	Estuarine	Brisban River, Moreton Bay	Australia	Pitt et al. (2009)
		13.0	Poultry farming	Effluent sample	Southeastern Gulf of California	Mexico	Pinon-Gimate et al. (2009)
Enteromorpha intestinalis	13.1–14.9		WWTP SE	Nearshore reef	Nanwan	Taiwan	Lin et al. (2007)
Flimanetous algae	15.0		BNR WWTP SE	Estuarine	Pine River, Moreton Bay	Australia	Pitt et al. (2009)
Ulva intestinalis	15.0	15.3–18.0	WWTP SE	Estuarine	Narragansett Bay, Rhode Island	USA	Thornber et al. (2008), Chaves (2004)
Catenella nipae	16.3–19.6		WWTP SE	Estuarine	Moreton Bay	Australia	Jones et al. (2001)
Flimanetous algae	17.0–19.0		BNR WWTP SE	Estuarine	Logan River, Moreton Bay	Australia	Pitt et al. (2009)
H. musciformis & U. fasciata	17.8		BNR WWTP SE	Sandy beach	Kalama Beach Park, South Maui	USA	This study
		>19.5	STE	Juptier Creek Monitoring Well #5	Southeast Florida	USA	Lapointe and Krupa (1995a)
Ulva fasciata	22.3		BNR WWTP SE	Basalt	Kahului WWRF, North-central Maui	USA	This study
Hypnea musciformis	25.6		Algae grown in 20% BNR WWTP SE	Laboratory Experiment	Lahaina WWRF, Maui	USA	(Dailer and Smith, submitted for publication)
Fucus vesiculosus	25.7		Anthropogenic	Estuarine	Scheldt River, Westerschelde Estuary	The Netherlands	Riera et al. (2000)
Ulva fasciata	30.3		Algae grown in 20% BNR WWTP SE	Laboratory Experiment	Lahaina WWRF, Maui	USA	(Dailer and Smith, submitted for publication)
Ulva fasciata	34.7		BNR WWTP SE	Sandy beach	South Kahekili Beach Park, West Maui	USA	This study
Ulva fasciata	43.3		BNR WWTP SE	Sandy beach	North Kahekili Beach Park, West Maui	USA	This study
Ulva fasciata	50.1		BNR WWTP SE via freshwater seep	Nearshore reef	North Kahekili Beach Park, West Maui	USA	This study

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The distinct δ^{15}N value of N sources allows for source determination in the marine environment through algal bioassays (Costanzo et al., 2005) despite the potential of isotopic fractionation by algal metabolism. Although phytoplankton have demonstrated strong isotopic preferences for ^{14}N over ^{15}N in N-rich conditions (Pennock et al., 1996), experiments with the macroalga *Enteromorpha intestinalis* determined that both ^{14}N and ^{15}N were taken up in N-rich conditions and this uptake was in proportion to the supply provided in the experimental treatments (Cohen and Fong, 2005). However if isotopic fractionation were to occur in algae under N-rich conditions, their resulting δ^{15}N value (‰) may be lowered by several parts per thousand and could possibly confound the interpretation of the results (Waser et al., 1998; Cole et al., 2004). In addition, the enzymatic process of N assimilation involving nitrate reductase may also affect algal δ^{15}N values (Mariotti et al., 1982).

Increasingly the view that algae incorporate new N from their environment with little to no isotopic fractionation or discrimination of source (anthropogenic or natural) is gaining support (Gartner et al., 2002; Cohen and Fong, 2005) especially in tropical settings where the natural sources of N are exceptionally low. Algal δ^{15}N values are likely to represent the integration of all available nitrogen sources over time scales of days to weeks. Such responsiveness allows for transplantation studies to determine the variety of N input into a coastline. For example, Costanzo et al. (2005) determined that algae expressed higher δ^{15}N values in a short time frame (~4 days) when collected from a natural area and relocated to a sewage affected location. Over the past decade, algal δ^{15}N values have increasingly been used in a variety of ecosystems across the world to successfully discriminate between anthropogenic and natural N sources and map the range of anthropogenic impact on alongshore and nearshore–offshore gradients (Lapointe, 1997; McClelland et al., 1997; France et al., 1998; Jones et al., 2001; 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; Table 1). The values of δ^{15}N for algae growing directly in front of sewage outfalls are often enriched with values ranging from 8‰ to 19‰ (Costanzo et al., 2001; Jones et al., 2001; Gartner et al., 2002; Barlie and Lapointe, 2005; Lin et al., 2007; Thornber et al., 2008; Pitt et al., 2009). Currently the highest reported algal δ^{15}N value is 25.7‰ from the heavily polluted (including sewage) Scheldt Estuary in The Netherlands (Riera et al., 2000). Because the process of denitrification releases N_2 into the atmosphere, some wastewater treatment plants use denitrification (in combination with nitrification) or Biological Nitrogen Removal (BNR) to reduce nitrogen levels in the wastewater (Wiesmann, 1994; Zumft, 1997). It is highly likely that facilities employing this method of N removal produce wastewater effluent with highly enriched δ^{15}N values.

Nuisance macroalgal blooms of *Hypnea musciformis* (Rhodophyta) and *Ulva fasciata* (Chlorophyta) are problematic in shallow coastal waters around many urbanized and agricultural regions of Maui, Hawai‘i. Beaches in bloom areas are regularly covered with extensive buildups of rotting algal biomass. In addition to obvious ecological impacts, these nuisance algal blooms cost the County of Maui $20 million US dollars annually as a result of clean-up costs and lost revenue due to reduced property values and occupancy rates in the city of Kihei alone (Van Beukering and Cesar, 2004). Recent research has determined that accelerated growth of *H. musciformis* and *U. fasciata* is driven by excess nutrients (Dailer and Smith, submitted for publication). Because of the proximity of the algal blooms to human population centers and agricultural regions on Maui, we hypothesized that the blooms are a result of sewage and agricultural pollution to shallow coastal regions.

The Clean Water Act (CWA) (also referred to as the Federal Water Pollution Control Act, 2002 as amended and codified at 33 U.S.C. Section 1251) is the primary federal law regulating anthropogenic sources of water pollutants, including nutrients. The CWA requires state water quality management and pollution control programs to have water quality goals (standards). The State of Hawai‘i water quality standards include criteria related to algal blooms expressed as numeric criteria for nutrients, turbidity, and chlorophyll *a* and narrative criteria requiring that state waters be free of substances attributable to domestic, industrial, or other controllable sources of pollutants which produce undesirable aquatic life. The State of Hawai‘i water quality standards for Class AA marine waters includes the support and propagation marine life, conservation of coral reefs, compatible recreation and aesthetic enjoyment (SH DOH, 2004). None of these goals are attained when a coast is subjected to algal blooms. If water quality standards are not attained, the waters are considered impaired, and the State of Hawai‘i Department of Health (SH DOH) and US EPA are required to determine the Total Maximum Daily Load (TMDL) for pollutants that are causing the impairment. A TMDL determines the maximum pollutant mass from all sources combined that can be discharged daily to a waterbody while still attaining water quality standards. A TMDL establishes a pollutant budget with wasteload allocations for point sources, load allocations for nonpoint sources; and a margin of safety.

TMDL = WLA + LA + MOS, where: WLA = wasteload allocation for point sources, LA = load allocation for nonpoint sources and MOS = margin of safety.

Through the determination of tissue δ^{15}N values of common algae, this study aimed to (1) identify coastal regions of anthropogenic N enrichment on the island of Maui via an island-wide coastline survey (2) use additional fine-scale surveys in identified areas of concern to map the extent of anthropogenic N along the coastline and (3) determine the extent of anthropogenic N across the coral reef adjacent to the highest δ^{15}N values found. An additional goal of this study was to determine the amount of effluent injected and corresponding nitrogen point source load estimates for the County of Maui Wastewater Reclamation Facilities over the past 11 years.

2. Study area

The island of Maui has a population of 143,574 (US Census Bureau 2008) and an annual visitor flux of approximately 2 million people (2,089,738 in 2008 http://hawaii.gov/dbedt/info/visitor-stats/ni-stats). The majority of the island remains in a relatively natural undeveloped state (the northwest and eastern regions). Wastewater on Maui is released primarily by underground disposal through shallow injection wells and cesspools. An injection well (IW) is a bored, drilled or driven shaft, or a dug hole, whose depth is greater than its largest surface dimension; an improved sinkhole; or a subsurface fluid distribution system used to discharge fluids underground (Code of Federal Regulations Chapter 40 Part 144.3). Cesspools are underground regions used for the disposal of human waste where untreated sewage is discharged directly into the ground. Leakage from cesspools can contaminate oceans, streams and groundwater by releasing disease causing pathogens and nitrates (http://www.epa.gov/region/water/groundwater/uic-hicesspools.html). Injection wells and cesspools are regulated by the US EPA under the authority of the Underground Injection Control (UIC) program, as provided by Part C of the Public Law 92-523, the Safe Drinking Water Act (SDWA) of 1974. The SH DOH administers a separate UIC permitting program under state authority. In addition, the SH DOH implements CWA water quality management planning (which includes establishing water quality

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Table 2
Island-wide collection sites and sampling distribution of macroalgae ($n = 3$ per genus per site).

Region	Collection site	*Ulva fasciata*	*Asteronema breviarticulatum*	*Hypnea musciformis*	*Ahnfeltiopsis concinna*
West Maui	Honokawai N	X		X	
	Honokawai	X		X	
	Honokawai S	X		X	
	Honolua N	X		X	
	Honolua S				X
	Kaanapali N			X	
	Kaanapali	*Acanthophora spicifera*			
	Kaanapali S	X		X	
	Kahana N	X		X	
	Kahana S	X		X	
	Kahekili BP N	X			
	Kahekili BP S				
	Kapalua E	X	X	X	
	Kapalua W		X		
	Lahaina Town N				X
	Lahaina Town S	*Acanthophora spicifera*			
	Laniopoko N	X		X	
	Laniopoko			X	
	Laniopoko S	X		X	
	Makahuna Gulch N		X		
	Makahuna Gulch		X		
	Makahuna Gulch S		X		
	Mala N	X		X	
	Mala		X		
	Mala S	X		X	
	Manuohule W	X	X		
	Manuohule		X		
	Manuohule E		X		
	Napili N		X		
	Napili	X	X	X	
	Napili S		X		
	Olowalu N		X		
	Olowalu			X	
	Olowalu S			X	
	Puumana N			X	
	Puumana S			X	
	Ukemehame N		X		
	Ukemehame S	*Acanthophora spicifera*			
	Waihikuli N	X			
	Waihikuli		X		X
	Waihikuli S				X
Central Maui	Baldwin E			X	
	Baldwin	X		X	
	Baldwin W			X	
	Hookipa BP E	X	X	X	
	Hookipa BP	X		X	
	Hookipa BP W			X	
	Kahului Harbor (break wall)				X
	Kahului Harbor				X
	Kahului N	*Acanthophora spicifera*			
	Kahului S	*Acanthophora spicifera*			
	Kahului WWTP			X	X
	Kite Beach E	X			
	Kite Beach	X			
	Kite Beach W			X	
	Maalaea 1	X		X	X
	Maalaea 2	X	X		
	Spreckelsville E	X			
	Spreckelsville	X		X	
	Spreckelsville W	X		X	
	Sugar Beach- Kealia Pond NWR N			X	
	Sugar Beach- Kealia Pond NWR S	X			
	Sugar Beach	X		X	
	Tavares E			X	
	Tavares W			X	
	Waiehu N	X			
	Waiehu S	X			
East Maui	Blue Pool 1		X		
	Blue Pool 2				X
	Haleakala NP 1	X	X		
	Haleakala NP 2	X	X		X
	Haleakala NP 3		X		
	Hana Bay 1	X	X		X
	Hana Bay 2		X	X	

(*continued on next page*)

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Table 2 (*continued*)

Region	Collection site	*Ulva fasciata*	*Asteronema breviarticulatum*	*Hypnea musciformis*	*Ahnfeltiopsis concinna*
	Keanae Point E				X
	Keanae Point W		X		
	Koki N		X		
	Koki S		X		X
	Nahiku 1		X		
	Nahiku 2		X		
	Nahiku 3		X		
	Venus Pools		X		
	Wainapanapa 1	X	X		
	Wainapanapa 2		X		X
South Maui	Ahihi Kinau	X			X
	Central Kihei N (Kalama BP)	X		X	
	Central Kihei	X			
	Central Kihei S	X			
	La Perouse 1 E	X	X		
	La Perouse 2		X		X
	La Perouse 3		X		
	La Perouse 4		X		
	La Perouse 5 W	X			
	Makena N	X			
	Makena	X	X	X	
	Makena S	X	X	X	
	Keawakapu BP	X	X	X	
	Wailea N	X	X	X	
	Wailea S	X		X	
	Waipulani BP N	X		X	
	Waipulani BP	X		X	
	Waipulani BP S	X		X	
Northwest Maui	Honokohau		X		
	Honokohau stream	*Cladophora sericea*			
	Kahakuloa		X		
	Punaha Gulch		X		
	Punaha Gulch N	X			
	Punaha Gulch S	X	X		
Southeast Maui	Arches 1		X		
	Arches 2		X		
	Big Kiawae 1		X		
	Big Kiawae 2	X			
	Kaupo 1		X		X
	Kaupo 2		X		

standards and performing TMDL studies) and holds the authority for the National Pollutant Discharge Elimination System (NPDES) permits in Hawai'i.

Most of the residents on Maui live in three main towns (Kahului, Kihei and Lahaina) that are served by centralized regional sewage collection and treatment systems. The County of Maui operates Wastewater Reclamation Facilities (WWRF) that use BNR followed by disposal into Class V injection wells (Parabicoli, pers. comm.) in Kihei (3 IWs), Kahului (8 IWs) and Lahaina (4 IWs). The majority of the injected wastewater at the WWRFs does not receive disinfection treatments (e.g. chlorine or ultra-violet radiation), nor does the SH DOH or US EPA require it at this time. The WWRFs are the three largest wastewater sources on Maui. Many smaller towns along the coastline adjacent to these major population centers use cesspools for sewage disposal. SH DOH and US EPA databases indicate that Maui has >6000 individual small septic or small cesspool wastewater systems (including those in the areas of Waiehu, Waihikuli and Maui Meadows) and more than 300 injection wells including large capacity septic (93) and wastewater treatment plants (59). Small individual sewage treatment plants with IWs are located in Kahului, Makena and Ma'alaea. Ma'alaea, located on the south-central coast, has one commercial and 12 condominium developments each with privately owned sewage treatment facilities and IWs. Ma'alaea also has two direct discharges to surface waters contributing low concentrations of N (from the Maui Ocean Center and Maui Electric Company) that are authorized under NPDES permits. Anthropogenic N loading on Maui also includes fertilizers from extensive agricultural operations that occur in the central portion of Maui between the north and south coast.

Kihei is a highly developed area in South Maui where algal blooms have persisted for decades (Wiltse, US EPA, pers. comm.). The extensive fringing reef adjacent to Kihei generally has poor water circulation so nutrients entering the reef flat are likely to have long residence times and/or be acquired by algae. In contrast, the reef in the Kahekili Beach Park (BP) area (near the Lahaina WWRF) lacks an extensive reef flat and generally has a persistent current flowing to the south (Storlazzi and Field, 2008). The shallow forereef (approximately 1.5–10 m offshore) has had algae blooms (primarily of *U. fasciata*) in the summers, when wave action from the north is diminished (pers. obs.). This area also frequently has bubbles of an unidentified gas flowing from the benthos and warmer-than-ambient-water freshwater seeps. The seeps are consistently present and are surrounded by rocks and coral rubble with black precipitates. The black precipitate is likely iron oxide which arises from anoxic conditions in the groundwater (Bhagat et al., 2004). This reef is located within the Kahekili Herbivore Fisheries Management Area (HFMA) that was established on July 25th 2009 by the State of Hawai'i, Department of Land and Natural Resources, Division of Aquatic Resources (DAR) (http://hawaii.gov/dlnr/dar/regulated_areas_maui.html). The Kahekili HFMA encompasses approximately 3.0 km of coastline and is now closed to the taking of herbivorous fishes and sea urchins in efforts to restore a healthy grazing population to combat excessive algal growth associated with the decadal documentation of coral decline (SH DLNR, 2006).

The SH DOH has reported to the US EPA and US Congress that the water quality in several coastal segments of Maui in the vicinity of the WWRFs, injection wells and injectate plumes are not

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meeting state water quality standards. Water quality impairments reported for the Kahekili area were due to exceeded water quality criteria for water column concentrations of Total Nitrogen (TN), Chlorophyll *a*, and Ammonia (Honokowai Point to Kaanapali), Total Phosphorous (TP) and turbidity (Honokowai BP) and turbidity at Kahekili BP. In addition, 19 coastal segments along the developed Kihei coast and three coastal segments of Kahului Harbor are currently listed as impaired for various combinations of pollutants including TN, Nitrite–Nitrate, Ammonia, TP, Chlorophyll *a* and turbidity. One segment of Kahului Harbor is listed as impaired due to exceedances of bacterial criteria (*Entercoccci*) (SH DOH, 2006).

3. Material and methods

3.1. Island-wide coastline survey

In the summer of 2007, an island-wide survey of intertidal algal δ^{15}N values from all accessible coastlines on Maui was conducted to locate areas and potentially identify sources of anthropogenic N enrichment. Maui has approximately 190 km of coastline with the majority of the population residing in a few discrete regions (Kahului, Waiehu, Kihei, Maalaea, Lahaina, Kaanapali, Kahana and Napili). Survey intervals occurred every 1.5 km in populated areas and every 8 km in unpopulated areas. Where possible, three sites 0.3 km apart were sampled per survey interval, intertidal macroalgae were sampled in triplicate per genera and two to three genera were collected when possible (from 45 sites, Table 2, Fig. 1a–f). The following macroalgae were collected during the survey: *Acanthophora spicifera*, *Ahnfeltiopsis concinna*, *Asteronema breviarticulatum*, *Cladophora sericea*, *H. musciformis*, and *U. fasciata* (Table 2). Using this approach, a total of 116 sites and 516 samples were collected around Maui; 21 km of coastline were inaccessible by foot due to treacherous terrain.

3.2. Fine-scale mapping survey

This survey aimed to identify the presence of sewage N along the coastline in areas with elevated δ^{15}N values and high recreational uses (Kahekili and Kalama BPs). All sampling occurred in the intertidal zone; sites extended along approximately 1.2 km of coastline centered on the highest δ^{15}N values found from the coastal survey (above) for Kahekili BP (near the Lahaina WWRF) and Kalama BP (adjacent to the Kihei WWRF) (Fig. 2). Naturally occurring, attached samples of *U. fasciata* were collected for δ^{15}N analyses (in triplicate per site, $n = 81$ and 96 for Kahekili and Kalama BPs, respectively) from sites approximately 100 m apart for the first five sites in the north, then every 50 m for the remainder of the sites to the south for Kihei; in Lahaina the last three southerly sites were 100 m apart (Fig. 2).

3.3. Mapping the Lahaina WWRF effluent plume with deployed algae

To determine the extent to which the effluent plume from the Lahaina WWRF stretched across the adjacent coral reef, we employed an approach similar to Costanzo et al. (2001), however we deployed samples of *U. fasciata* ($n = 96$ per deployment) 0.5 m from the benthos. In January 2009, 32 semi-permanent

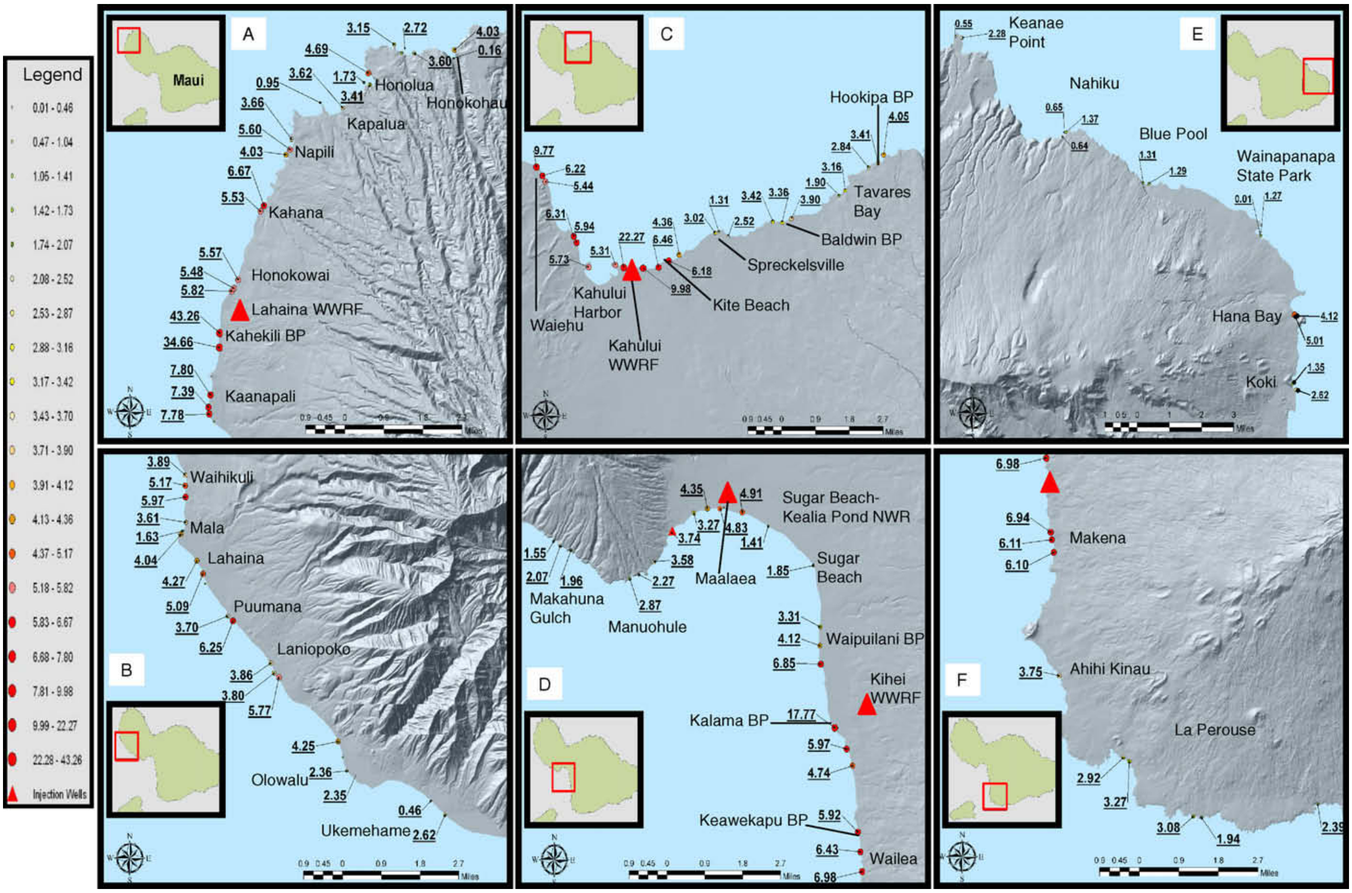

Fig. 1. Island-wide algal collection sites and associated average algal δ^{15}N values from the northwest (a), southwest (b), north-central (c), south-central (d), northeast (e) and southeast (f) regions of Maui. Injection well locations are represented by red triangles.

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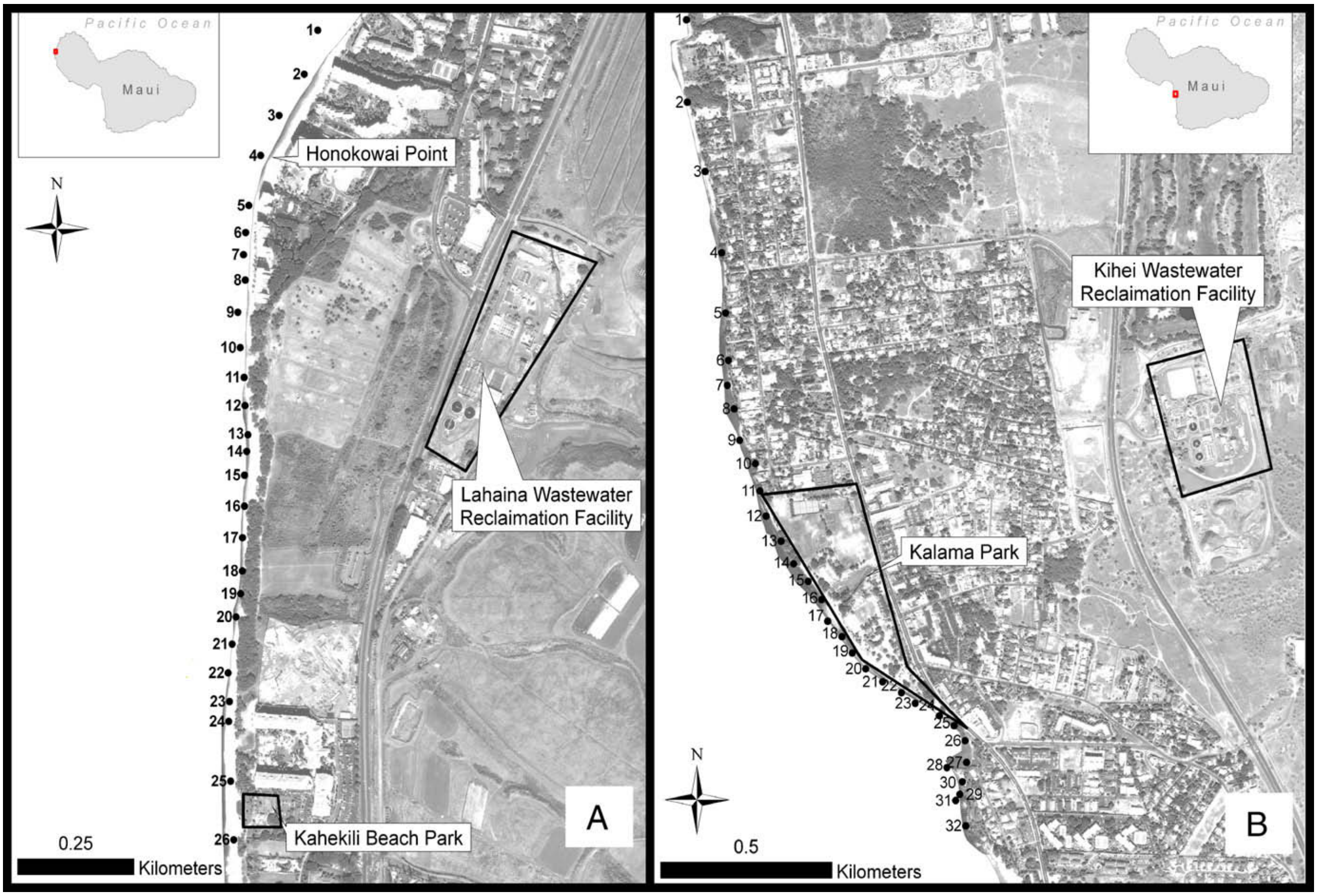

Fig. 2. Fine-scale mapping survey collection sites of *Ulva fasciata* labeled from north to south for the Kahekili (A, 1–27) and Kalama (B, 1–32) Beach Park areas. (NAD 1983, UTM Coordinate System, Quickbird© Imagery, 2007.)

sites (Fig. 3) were installed spanning the entire coral reef adjacent to sites 16–27 (Fig. 2a). This area was chosen because of our goal to examine the presence of the effluent on the coral reef and the area to the north (sites 7–15, Fig. 2a) lacks coral reef formation. To address the presence of effluent on the reef we installed six transects (T1–T6) each with four sites (A–D) at the following depths (m): 1.5 (A), 2 (B), 3 (C) and 6 (D) (Fig. 3). An additional eight sites (S1–S8) were installed in the shallow zone containing warmer-than-ambient-freshwater seeps (at 1.5 m depth) (Fig. 3). Samples of *U. fasciata* were first acclimated to low nutrient seawater for seven days to deplete internal N stores by housing individual samples in 1.0 L beakers with aeration lines in water baths to prevent the seawater from heating and the seawater was changed every two days. Acclimated samples were then housed in 10 × 10 cm cages enclosed in plastic mesh and attached to float lines hovering approximately 0.5 m from the benthos. T3B was located directly over a warm freshwater seep and an additional seep site (NS) was added in May to the north of T2B. In collaboration with DAR, additional samples were deployed at the island of Molokini, a State of Hawai'i Natural Area Reserve located offshore of south Maui, to observe changes in algal δ^{15}N values when deployed in an area of low anthropogenic impact.

3.4. Algal sample preparation

Samples were prepared in triplicate per collection site (per genus for the coastline survey) for tissue δ^{15}N analysis. For deployed samples, field and acclimated samples were prepared in triplicate to obtain the initial and acclimated δ^{15}N values. Samples were rinsed in deionized water, dried at 60 °C to a constant weight, ground with mortar and pestle into a powder and sent for mass spectrometer analysis to the Biogeochemical Stable Isotope Laboratory, University of Hawai'i at Mānoa, for tissue δ^{15}N determinations. A small portion of the samples from the fine-scale mapping survey were sent to the USGS Reston Stable Isotope Laboratory, Reston Virginia. Samples were weighed then analyzed with a Carlo Erba NC2500 Elemental Analyzer, Finnigan MAT ConFloII, and Finnigan MAT DeltaS. Ratios of ^{15}N:^{14}N were expressed relative to atmospheric nitrogen and calculated as:

$$\delta^{15}\mathrm{N}(‰) = \{(R_{\mathrm{sample}}/R_{\mathrm{standard}}) - 1\} \times 10^3$$

where $R = {}^{15}\mathrm{N}/{}^{14}\mathrm{N}$ (Sweeney et al., 1978).

3.5. Statistical analyses

The island-wide survey contained 40 and 5 sites where two and three genera were collected, respectively (Table 2). Additional collections per site were made to determine if different genera expressed different δ^{15}N values from the same location, although previous studies suggested that algal δ^{15}N values were not affected by algal species or physiology (Umezawa et al., 2002; Derse et al., 2007) and other studies have homogenized or used the mean δ^{15}N of all collected algae per site with no explanation of potential differences among genera (Steffy and Kilham, 2004; Barlie and Lapointe, 2005). Correlations were performed among the δ^{15}N

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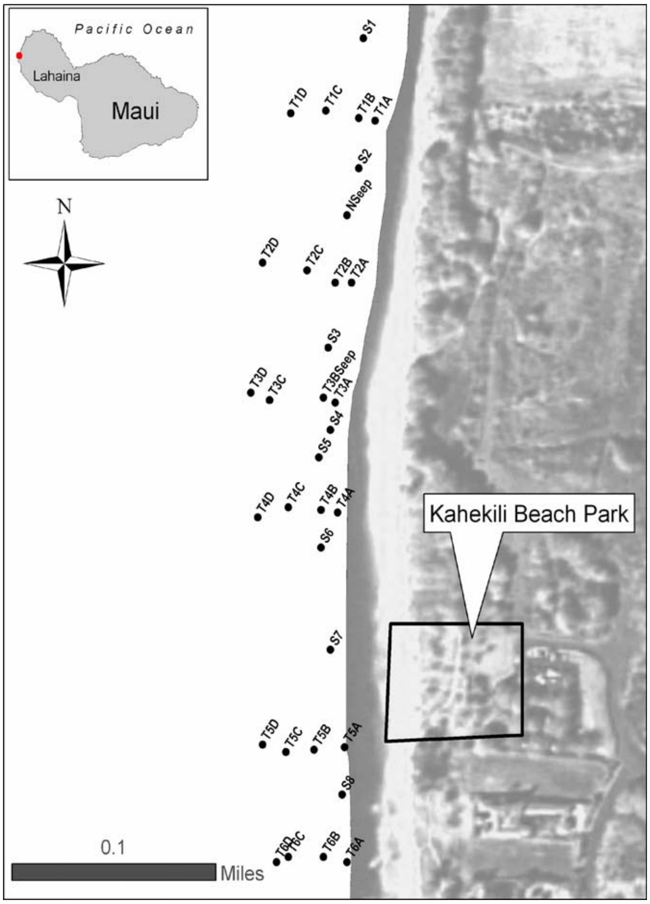

Fig. 3. Algal deployment sites to determine the expansion of the Lahaina WWRF effluent plume across the Kahekili area. (NAD 1983, UTM Coordinate System, Quickbird© Imagery, 2007.)

values of genera collected from the same sites to determine if the values were representative (Moore and McCabe, 2002). The $\delta^{15}N$ values of genera from the same site were significantly and strongly correlated demonstrating the similarity of expressed values among genera collected from the same site (see Section 4). All $\delta^{15}N$ values of collected samples per site were pooled for subsequent analyses. Data from the island-wide survey were normally distributed and had unequal sample sizes per site (n) (because multiple genera were collected from 45 sites). To determine if the $\delta^{15}N$ values were significantly different between sites, data were analyzed with a one-way ANOVA with the algal $\delta^{15}N$ value as the dependant variable and site as the categorical factor. After a significant result was determined ($P < 0.00001$), an Unequal n post hoc test was performed to determine significance within sites.

Data from the fine-scale mapping surveys were normally distributed and the variances were homogeneous. These data were analyzed with a one-way ANOVA with the algal $\delta^{15}N$ value as the dependant variable and site as the categorical factor. When significant results were detected ($P < 0.00001$), Tukeys post hoc tests were used to determine significant levels within factors. Data from the deployed algal samples were not normally distributed and had unequal sample sizes per site by the end of the deployments due to unexpected wave events in which samples disintegrated. These data were analyzed with a General Linear Model with Type III error, after a significant result (see below for results by deployment) an Unequal n post hoc test was performed to determine significance of algal $\delta^{15}N$ values within deployment sites and initial levels (acclimated and field collected samples). All statistical tests were performed with Satistica 6.0.

3.6. GIS analysis Lahaina WWRF effluent plume

A Geographic Information System (GIS) was used to generate an inverse weighted distance (IDW) algorithm of the algal $\delta^{15}N$ values deployed at Kahekili BP in May. A Garmin GPS 76CS Plus was used to obtain the GPS coordinates in WGS 84 of the algal deployment sites. GPS points were converted to a grid in ARCGIS Spatial Analyst. The ESRI GRID modeled the presence of effluent and possible effluent dispersion across the Kahekili reef using algal $\delta^{15}N$ values. This dataset depicts the geographic distribution of the averaged $\delta^{15}N$ value from 33 sample points in the nearshore and offshore central reef in the Kahekili HFMA. This dataset: (1) only accounts for surface area, (2) had a resolution of 1.33 m ESRI GRID and (3) encompasses an area of approximately 194,334 m^3.

3.7. County of Maui WWRF injection well nitrogen loading estimate

Annual water reuse and injectate rates provided by the County of Maui, Department of Environmental Management, from 1997 to 2008 were used to determine order of magnitude Total Nitrogen Load (TNL) estimates for the combined injectate of the Lahaina, Kihei and Kahului WWRFs. Monthly average effluent flow rates,

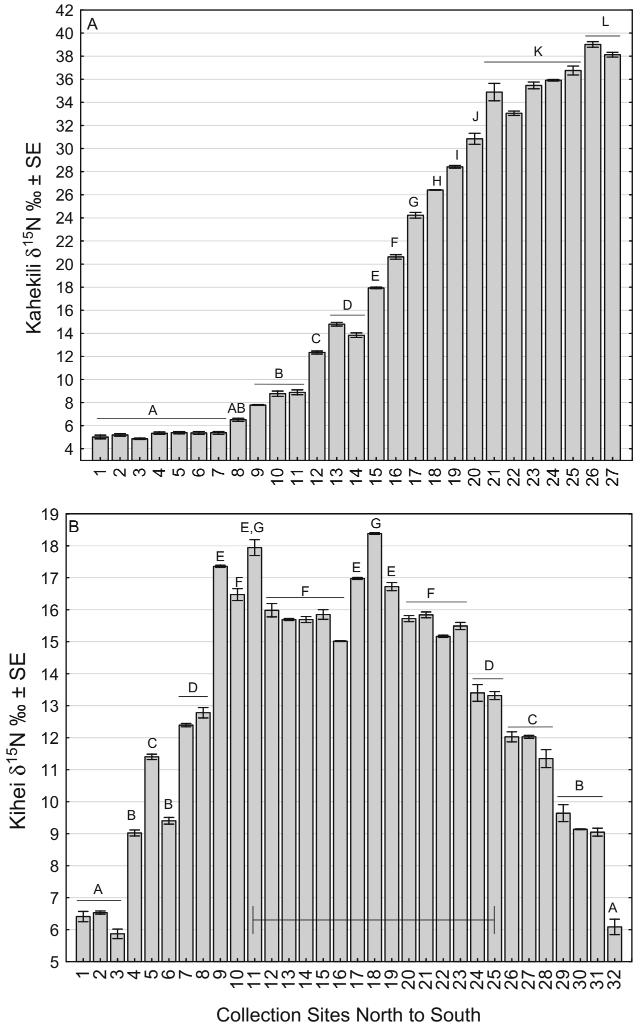

Fig. 4. Average *Ulva fasciata* $\delta^{15}N$ values from the fine-scale mapping surveys in the areas of (A) Kahekili Beach Park (BP) and (B) Kalama BP; sites 11–25 encompass Kalama BP (represented by the line). Significant differences are represented by different letters.

percent of effluent reuse and monthly average TN concentration for the period from 2006 to 2008 were used to estimate the daily and annual TNL of the wastewater injectate from the Lahaina, Kihei and Kahului WWRFs. The "load" or mass of a chemical entering or leaving an area is the product of the volume of water that the chemical is using as its transport medium and the concentration of the chemical in the water (Rice and Izuino, 1998):

$$\text{Load(mass)} = \text{Concentration (mass/volume or mass/mass)} \times \text{flow (volume or mass)} \quad (1)$$

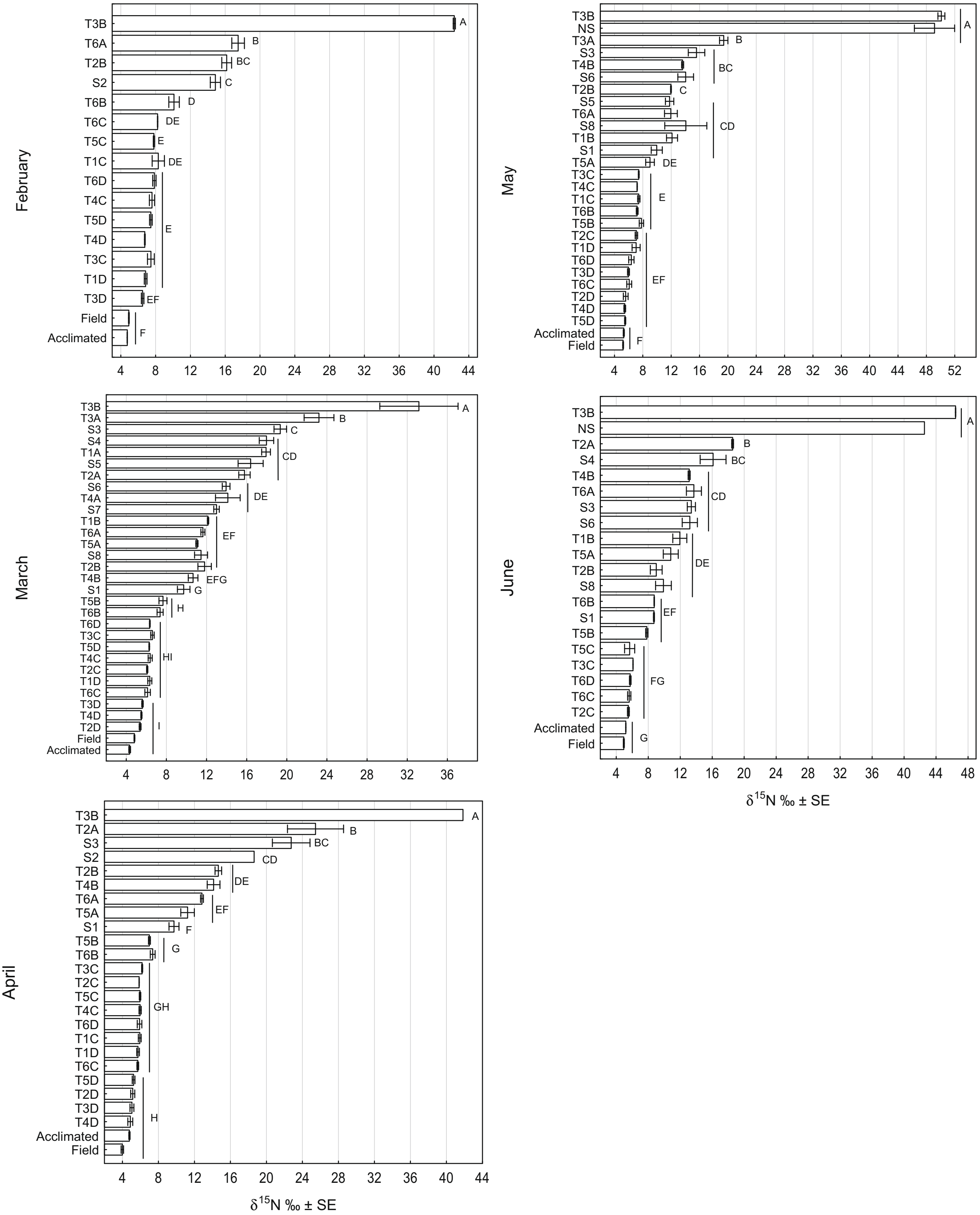

Fig. 5. February through June 2009 average δ^{15}N values of *Ulva fasciata* for field, acclimated and deployed samples at Kahekili (at the respective sites). Significant differences are represented by different letters.

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Estimated daily TNL or mass flux was calculated with wastewater effluent TN concentration data and measured or estimated injectate flow rates:

TNL (kg d^{-1}) = [(N concentration, mg L^{-1}) $*$ (Flow rate, $m^3\ d^{-1}$)]/1000, where, kg d^{-1} = kilograms per day; mg L^{-1} = milligrams per liter; $m^3\ d^{-1}$ = cubic meters per day.

Wastewater treatment and pollution control programs in the US typically express concentration in milligrams per liter (mg L^{-1}), volumetric flow rate in million gallons per day (10^6 gal d^{-1}) and pollutant loads in pounds per day (lbs d^{-1}). Daily TNL estimates were calculated with wastewater effluent TN concentration data and measured or estimated injectate flow rates according to Rice and Izuino (1998):

TNL (lbs d^{-1}) = (N concentration, mg L^{-1}) $*$ (Flow rate, 10^6 gal d^{-1}) $*$ 8.34, where 8.34 is the factor accounting for conversion from metric (mg, L) to English (lbs, gal) units: 8.34 = mg $L^{-1} \times 0.001$ g $mg^{-1} \times 0.002203$ lbs $g^{-1} \times 3.785$ L $gal^{-1} \times 10^6$ gal d^{-1}.

4. Results

4.1. Island-wide coastline survey

Multiple common genera from the major macroalgal divisions were collected from 45 sites to determine if differences in δ^{15}N values occurred among algae from the same site. Differences (or variability) of δ^{15}N values in algae at the same site could arise from physiologically different nitrogen uptake rates and storage capacities (Wallentinus, 1984) and pigment complexes because phycobilin pigments in the Division Rhodophyta require more nitrogen atoms than other pigments (Graham and Wilcox, 2000). However, strong (r close to 1.0) significant ($P < 0.05$) correlations were found between pairs of all genera collected from the same locations (*U. fasciata* δ^{15}N = 0.024 + 1.01* *H. musciformis* δ^{15}N, $r = 0.97$, $n = 75$; *U. fasciata* δ^{15}N = 0.022 + 1.02* *A. breviarticulatum* δ^{15}N, $r = 0.96$, $n = 42$; *H. musciformis* δ^{15}N = 1.09 + 0.809* *A. breviarticulatum* δ^{15}N, $r = 0.96$, $n = 24$; *A. breviarticulatum* δ^{15}N = −0.030 + 0.913* *A. concinna* δ^{15}N, $r = 0.94$, $n = 19$; *H. musciformis* δ^{15}N = −1.72 + 1.18* *A. concinna* δ^{15}N, $r = 0.99$, $n = 8$). This revealed that the isotopic signatures were representative of each other (Moore and McCabe, 2002) and the data from these sites were expressed as the average algal δ^{15}N value. Average algal δ^{15}N values significantly varied by site ($F_{120,386} = 402.99$, $P < 0.00006$). Generally, algae collected from areas with low anthropogenic impact had low δ^{15}N values (Makahuna Gulch, Manuohule, Ukemehame and La Perouse, Fig. 1b, d and f). The lowest δ^{15}N values were found in algae collected from east and southeast Maui, ranging from 0.009‰ to 1.62‰ (Wainanpapa State Park, Kaupo, Kaenea Point, Nahiku, Blue Pool, Koki, Haleakala National Park and Venus Pools; Fig. 1e). The highest average algal δ^{15}N value within areas of low impact was from Arches in southeast Maui at 4.02 ± 0.05‰. This value was significantly lower ($P < 0.004$) than those of algae collected from sites adjacent to the urban areas of Waiehu, Kahului, Kahului Harbor, Kite Beach, Keawakapu BP, Wailea, Makena, South Waipuilani BP, and Kaanapali (Fig. 1 a, c, d and f).

The highest δ^{15}N values in this study were found among algae collected adjacent to the County of Maui's Wastewater Reclamation Facilities (WWRF) at two sites in Lahaina (near Kahekili BP) (35.7 ± 0.05‰ and 43.3 ± 0.08‰), Kahului (22.3 ± 0.97‰) and Kihei (at Kalama BP) (17.8 ± 0.04‰) (Fig. 1a, c and d). These sites were significantly different from each other and significantly higher than all other sites ($P < 0.00004$). The δ^{15}N values of algae collected

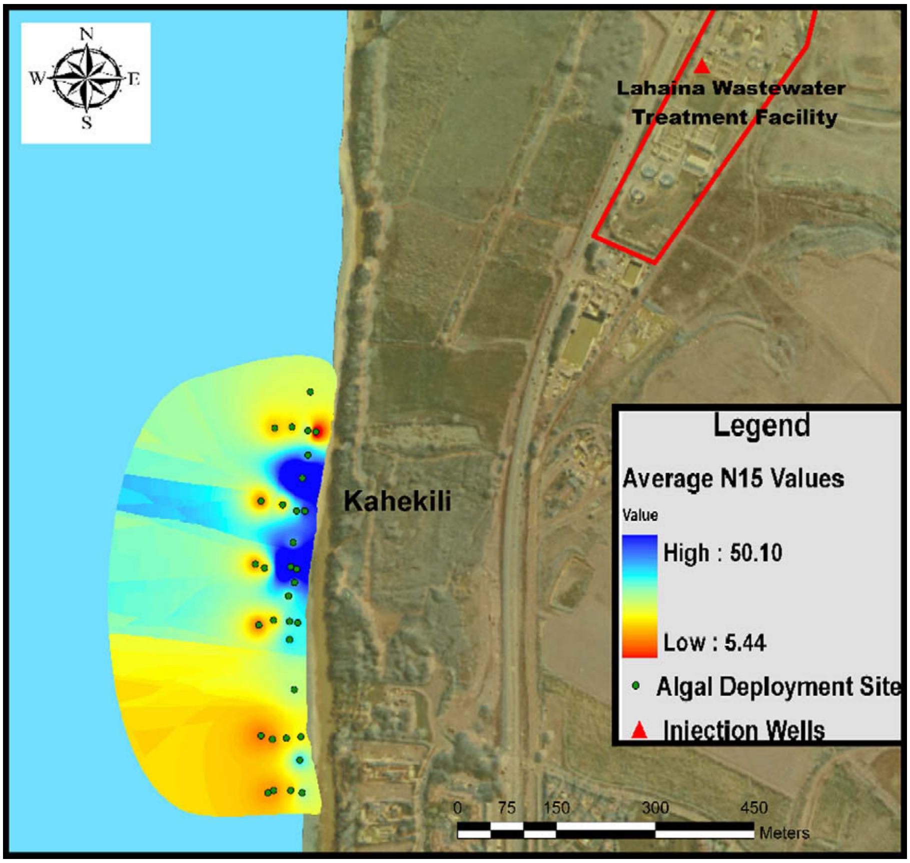

Fig. 6. Inverse weighted distance algorithm (IDW) of the algal δ^{15}N values deployed at Kahekili Beach Park in May 2009. (NAD 1983, UTM Coordinate System, United States Geological Survey Orthoimagery, 2003.)

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adjacent to the Kahului WWRF reservoir and the residential area of Waiehu with cesspools, were 10.0 ± 0.06‰ and 9.8 ± 0.08‰, respectively, and were not significantly different from each other but were significantly different from all other sites ($P < 0.00004$; Fig. 1c). Algal blooms of *H. musciformis* and *U. fasciata* were observed at most of the impacted sites (above); however, multiple algal species were blooming at the Kahului WWRF, Kite Beach and Keawakapu BP. Algal blooms were not observed in Makena where sand is the dominant benthic substrate, which would prevent algae from attaching to the benthos. In addition, massive algal blooms (primarily of *H. musciformis*) were observed in central Maui across the north coast from Spreckelsville to Hookipa BP which is adjacent to extensive agricultural operations. The algal δ^{15}N values across this region were low, ranging from 2.0‰ to 3.5‰ (Fig. 1c).

4.2. Fine-scale mapping survey

A significant effect of site was determined for the coastal surveys in Kahekili ($F_{26,57} = 2561.3$, $P < 0.00001$) and Kihei ($F_{31,64} = 616.83$, $P < 0.00001$). The seven most northerly sites for the Kahekili BP survey were located on Honokowai Point (directly adjacent to the Lahaina WWRF) where a shallow (<0.5 m depth) intertidal region extends offshore for ∼100 m (Fig. 2). The algal δ^{15}N values from these sites ranged from 5.0 ± 0.10‰ to 5.4 ± 0.08‰ which were significantly lower than sites 9–27 to the south ($P < 0.0002$, Fig. 4a). The algal δ^{15}N values from sites 9–27 increased to the south, ranging from 7.8 ± 0.03‰ to 39.1 ± 0.06‰ with highly significant differences ($P < 0.0002$) between most collection sites (Fig. 4a). Collections sites 12 through 27 were notably deeper (∼1.5 m) than sites 1–11 in the north, even though distance from shore was unchanged. In Kihei, the algal δ^{15}N values from sites 1–3 (in the north) and site 32 (in the south) ranged from 5.9 ± 0.09‰ to 6.5 ± 0.03‰. These values were significantly lower than sites 4 through 31 ($P < 0.00015$, Fig. 4b). The algal δ^{15}N values increased towards Kalama BP (sites 11 through 25) at sites 4–8 (north to south) and 31–26 (south to north) ranging at these sites from 9.0 ± 0.06‰ to 12.8 ± 0.10‰ (Fig. 4b). The highest algal δ^{15}N values were found at sites 9 through 23 (centered on Kalama BP), ranging from 15.0 ± 0.01‰ to 18.4 ± 0.02‰, which were significantly higher than all other sites ($P < 0.00015$).

4.3. Mapping the Lahaina WWRF effluent plume with deployed algae

Every month for one week from February through June 2009, acclimated samples of *U. fasciata* ($n = 3$ per site at 32 sites, 96 total) were deployed at sites across the Kahekili area (Fig. 3). Over the five month period, a total of 480 samples were deployed, however only 344 samples were recovered, processed and analyzed for tissue δ^{15}N due to unforeseen circumstances. Many samples were subjected to large swell events that disintegrated the samples; sites with only one recovered sample were not included in statistical analyses. A significant effect of site was found for each deployment (GLM ANOVA: February, $F_{16,27} = 517.5$, $P < 0.0000$; March, $F_{30,60} = 79.31$, $P < 0.0000$; April, $F_{24,36} = 105.88$, $P < 0.0000$; May, $F_{27,47} = 144.67$, $P < 0.0000$; June, $F_{21,27} = 278.04$, $P < 0.0000$). Regardless of the deployment month, all samples deployed over freshwater seeps drastically and significantly increased ($P < 0.0002$) in tissue δ^{15}N values ranging across deployments from lowest in March (33.1 ± 2.9‰) to the highest in May (50.1 ± 1.7‰) (Fig. 5). Also across all deployments, significantly increased ($P < 0.02$) algal δ^{15}N values were observed throughout the shallow region (1.5–2.0 m, nearshore sites S1–S8 and Transect sites A and B; Fig. 5). Significantly higher than initial tissue δ^{15}N values were repeatedly found at T6A which is 345 m to the south of the freshwater seep site T3B ($P < 0.0002$, Fig. 5). In addition, the values from algae deployed in the shallow sites to the south were consistently higher than those from S1 in the north (Fig. 5). The inverse weighted distance (IDW) algorithm of the May deployment agrees with the results above that the majority of the shallow region at Kahekili is affected by the Lahaina WWRF effluent plume (Fig. 6).

The greatest number of samples was recovered in March when conditions were calm throughout the entire duration of the deployment. The February deployment had the least amount of samples recovered due to a large storm that generated 1 m wind swell. However, this was the only deployment where the δ^{15}N

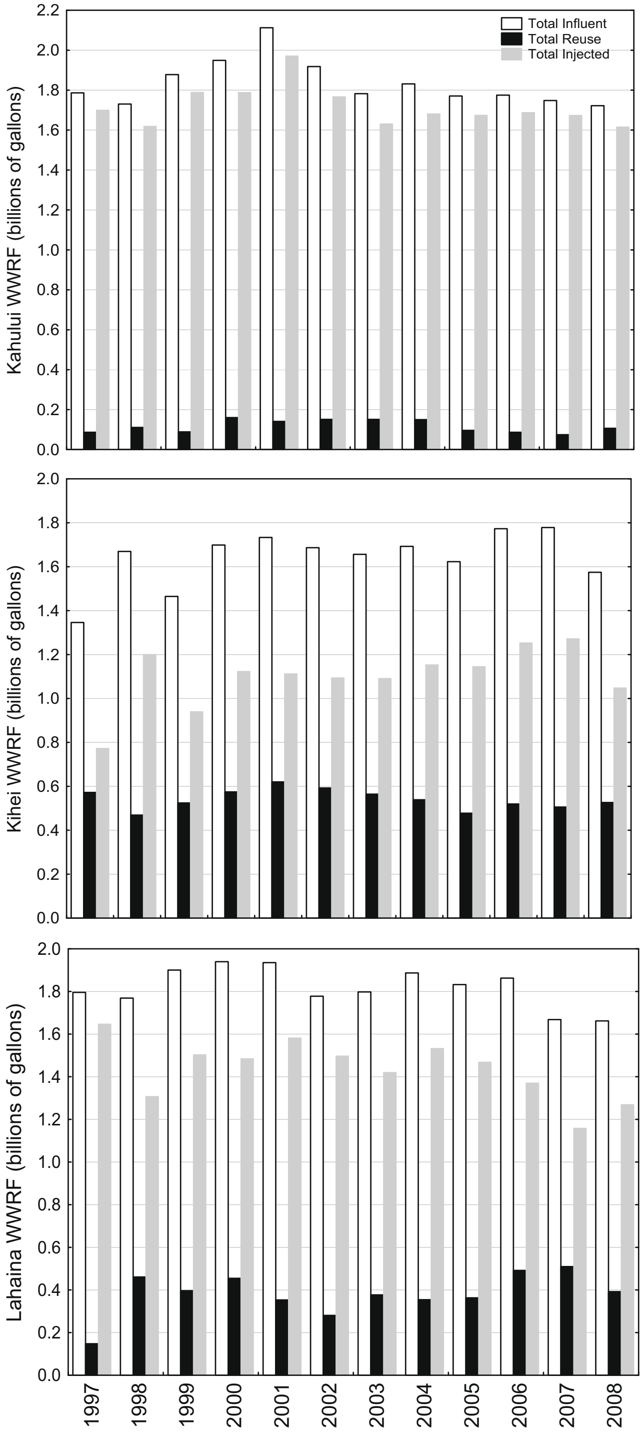

Fig. 7. 1997–2008 Calculations of annual influent (white bars), reused effluent (black bars) and injected effluent (gray bars) for the Kahului, Kihei and Lahaina Wastewater Reclamation Facilities (WWRF).

values of samples located ~100 m offshore at ~6 m depth (T6D, T5D and T4D) significantly increased ($P < 0.04$) from initial values (Fig. 5). In general, $\delta^{15}N$ values of samples at Transect sites C and D (3–6 m depth) were increased but not significantly elevated from initial values (with the exception of the February deployment) (Fig. 5). Samples deployed at Molokini in May for seven days did not change in $\delta^{15}N$ from their initial values (initial: 4.7 ± 0.06‰, final: 4.4 ± 0.02‰, $P = 0.75$).

4.4. County of Maui WWRF injection well nitrogen loading estimate

In 2008, the average daily flows through injection wells for the Lahaina, Kihei and Kahului WWRFs were 12,900, 9,600, and 16,800 $m^3\,d^{-1}$ (or 3.4, 2.5 and 4.4 million gal d^{-1}), respectively. From 1997 to 2008, over 3.78 million m^3 (1.0 billion gal) of effluent was injected per facility annually (with the exception of the Kihei WWRF in 1999; Fig. 7). Over an 11 year period, the Lahaina, Kihei and Kahului WWRFs combined have injected more than 193 million m^3 (51 billion gal) of secondarily treated effluent. Assuming an average TN effluent concentration of 7 mg L^{-1} for Lahaina and Kihei, and 12 mg L^{-1} for Kahului (where there is less nitrogen removal), an order of magnitude estimate of 1.74 million kilograms (3.84 million lbs) of TN have also subsequently been injected. The percentage of reused effluent over the same time frame for the Lahaina, Kihei and Kahului WWRFs was 21%, 33% and 6%, respectively (Fig. 7). From 2006 to 2008, the daily TNL of the Lahaina, Kihei, and Kahului WWRFs injectate ranged from 79 to 97 kg (174–215 lbs), 59–89 kg (131–196 lbs) and 174–207 kg (384–457 lbs) of N d^{-1}, respectively. The annual TNL of the Lahaina WWRF injectate ranged from 28,873 to 35,530 kg (63,609–78,274 lbs) of N yr^{-1}. The annual TNL of the Kihei WWRF injectate ranged from 21,676 to 32,525 kg (47,754–71,654 lbs) of N yr^{-1}. The annual TNL of the Kahului WWRF injectate ranged from 63,539 to 75,672 kg (139,978–166,705 lbs) of N yr^{-1}. Our work suggests that a substantial amount of this loading traveled to the nearby coastal zones.

5. Discussion

Foliose and filamentous macroalgae are excellent indicators of nitrogen sources in marine environments, because they (1) have high nutrient uptake rates and therefore quickly respond to pulses of nutrients (Wallentinus, 1984) (2) acquire and integrate all sources of water column nutrients over extended periods of time (Costanzo et al., 2005; Cohen and Fong, 2005; Lin and Fong, 2008), (3) are attached to the benthos and therefore represent a specific area and potential relationships with regional submarine groundwater discharge, (4) are easily collected and prepared for analysis, (5) require minimal cost and effort to perform analytical procedures to determine $\delta^{15}N$ values and (6) can be collected from one area and deployed in affected or control areas over minimal time scales to examine dominant source(s) of N in the area. Source identification through algal $\delta^{15}N$ values maybe complicated with the presence of multiple nitrogen sources with distinct signatures and biogeochemical processes that alter isotopic composition (Kendall, 1998). Although no algal 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). In addition, enzymatic processes involving the assimilation of N may also lower the $\delta^{15}N$ value as metabolism may show preference for lighter isotopes (Mariotti et al., 1982).

Despite these possible complications, algal $\delta^{15}N$ values have been used globally to detect sources of anthropogenic N in coastal environments. Gartner et al. (2002) document that (1) algal $\delta^{15}N$ values provide a useful means of tracing sewage that is more sensitive than conventional methods and (2) $\delta^{15}N$ values of *Ulva australis* reflect exposure to effluent in less than seven days (through laboratory experiments with sewage effluent). In Taiwan, Lin et al. (2007) document that algal $\delta^{15}N$ values successfully trace sewage effluent year round and that this method is better at detecting anthropogenic N loading than the tissue N contents and C/N ratios. In all cases where algal tissue has been used to trace sewage input into coastal environments, $\delta^{15}N$ values nearest sewage outfalls or treatment facilities are elevated relative to natural background signatures. Further, the average algal $\delta^{15}N$ values reported from sewage impacted areas from around the world range from 4‰ to 25.7‰ (Table 1).

The algal $\delta^{15}N$ values from the Maui coastline survey ranged from 0.009 to 43.3‰. With the exception of the values in close proximity to the Lahaina WWRF (43.3 ± 0.08‰) all values were within the range of globally reported algal $\delta^{15}N$ values from respective impacted and background sites (Table 1). All surveys and deployments in this study detected highly elevated algal $\delta^{15}N$ signatures (17.8‰ to 50.1‰, Table 1) in areas proximal to WWRFs with Class V sewage injection wells, demonstrating that the injected effluent from the WWRFs in Lahaina, Kihei and Kahului flowed into the nearshore marine environment. In addition, the Maui coastline survey revealed algal $\delta^{15}N$ values significantly higher than background levels ranging from 6‰ to 8‰ in coastal urbanized areas of Maui. These values compare well with those reported for of anthropogenic N enrichment from across the world (Table 1). Potential sources of N enrichment in these areas may include runoff and/or groundwater pollution from the use of reclaimed water for irrigation (e.g. Makena and Kaanapali golf courses) or leakage from septic tanks and/or cesspools (e.g. Waiehu, Waihikuli and Maui Meadows, Table 1).

Ma‘alaea is small coastal development adjacent to extensive sugarcane fields with 12 secondary sewage injection wells ranging in depth from 12.1 to 18.3 m and one that is 88.4 m. Substantial declines in percent coral cover has occurred in this area from 50–75% to 8% over the past decade (SH DLNR, 2006) as persistent algal blooms of *H. musciformis*, *U. fasciata* and *A. spicifera* have proliferated. The combination of two distinct N sources (fertilizer and sewage N) complicates the interpretation of the expressed algal $\delta^{15}N$ value because (1) the average of the two N sources may be expressed and/or (2) the probability of fractionation would increase, assuming that the area is a N-rich environment, which would lower the $\delta^{15}N$ values (Kendall, 1998). Therefore, to understand the changes in algal $\delta^{15}N$ values under these circumstances, further experimentation of growing algae in a range of combinations of sewage and fertilizer N (e.g. treatments of 5% fertilizer and 15% sewage N and vice versa) with the same initial concentrations of N species in both sources is needed to determine which source is more prevalent in the Ma‘alaea area. Lastly, massive algal blooms (primarily of *H. musciformis*) were observed during the study in central Maui across the north coast from Spreckelsville to Hookipa BP which is adjacent to extensive agricultural operations. The algal $\delta^{15}N$ values across this region were low ranging from 2.0‰ to 3.5‰ supporting the view that agricultural N is driving that regional bloom (Table 1).

Additional fine-scale mapping surveys along the coastline in areas of high recreational uses and heavy algal $\delta^{15}N$ signatures at Kahekili BP and Kalama BP revealed consistent results that wastewater effluent was present in the coastal environment in both areas of the WWRFs with Class V injection wells. Algal $\delta^{15}N$ values from the fine-scale mapping survey in Kihei revealed a pattern that confirms an effluent plume model generated by USGS (Hunt, 2006) which predicts that the Kihei WWRF effluent plume spans 1 km of coastline centered on Kalama BP, subsequently impacting less of the coastal region to the north and south. Algal $\delta^{15}N$ values were

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significantly lower in the far north and south endpoints of the survey than those from the sites near Kalama BP, where significantly elevated algal δ^{15}N values spanned ~1 km of coastline.

Further, the fine-scale mapping of Kahekili BP shoreline identified the presence of effluent in the nearshore marine environment slightly to the south of the Lahaina WWRF spanning at least 1.2 km of coastline. Coral cover in this area has declined from 55% to 33% over the past decade as algal (invasive and native) abundance in the area has increased (SH DLNR, 2006). To combat increased algal growth and subsequent coral decline in the Kahekili Herbivore Fisheries Management Area (HFMA) was established where the taking of herbivores is prohibited (http://hawaii.gov/dlnr/dar/regulated_areas_maui.html). The algal δ^{15}N values from the sites in the north, directly adjacent to the Lahaina WWRF on Honokowai Point, in <0.5 m depth ranged from 5.0‰ to 5.4‰ which were significantly lower than the values of algae collected from sites to the south in ~1.5 m depth even though distance from shore was unchanged. The results from this survey determined that the plume of effluent from the Lahaina WWRF flowed to the south of the facility, suggesting that the prominent landmass of Honokowai Point could be diverting the effluent to the south.

To determine the extent of the Lahaina WWRF effluent plume across the coral reef at Kahekili BP, we deployed samples of *U. fasciata* at 32 sites spanning the area five times from February through June 2009. Regardless of the month of deployment, all samples deployed over freshwater seeps drastically and significantly increased in δ^{15}N values (from ~5.0‰ to 33.1–50.1‰). These values currently represent the highest δ^{15}N values ever reported from algal tissue; the highest value reported in the scientific literature is that of *Fucus vesiculosus* collected from the Scheldt River, Westerschelde Estuary in The Netherlands, at 25.7‰ (Riera et al., 2000; Table 1). The high δ^{15}N values from algal deployments were higher than the values from algae grown in effluent from the Lahaina WWRF (Table 1). The increased difference in δ^{15}N values may be attributable to further denitrification occurring as the effluent is transported to the ocean and/or variability in the δ^{15}N values of the effluent itself. Further rigorous testing of the Lahaina WWRF effluent is needed to address the variability of the source δ^{15}N value to determine if (1) the source values can be as high as those observed in algal deployments and/or (2) further denitrification is occurring during subterranean transport from the WWRF to the ocean. Significant increases in algal δ^{15}N values were observed throughout the nearshore shallow region including sites 345 m to the south of the freshwater seeps, regardless of deployment. These results confirm that the injected effluent from the Lahaina WWRF is continuously flowing through the reef at Kahekili and then subsequently flows to the south. The increased values to the south are consistent with the findings from the fine-scale mapping survey and research conducted by USGS of wind patterns, wave activity and current direction, which found that the prevailing nearshore current in this area flows from north to south (Storlazzi and Field, 2008). A large storm generated 1 m wind swell during the February deployment which was the only deployment where the δ^{15}N values of samples located ~100 m offshore at ~6 m depth significantly increased from initial values indicating that the effluent is detectable at these sites during large scale mixing events. Generally, the δ^{15}N of samples deployed in 6 m depth were increased but not significantly elevated from initial values. Because the Lahaina WWRF effluent plume is freshwater and more buoyant than saltwater, future studies will include additional deployments of samples closer to the surface of the water at offshore sites to determine if the plume extends ~100 m offshore but remains undetected at 6 m depth until large scale mixing events occur, such as the storm in February 2009.

The evidence of sewage effluent from the Lahaina WWTP encompassing the nearshore marine environment that is used for recreation potentially poses serious threats to human health because of the associated microbial (bacterial and viral) assemblages normally found in sewage (Tree et al., 2003). Sewage effluent can be successfully disinfected with ultraviolet light (UV, 254 nm) irradiation which kills more than 99% of coliform, fecal coliform, fecal streptococci and heterotrophic bacteria (Oliver and Cosgrove, 1975). In 2008, the Lahaina WWRF processed an average of 3.4 million gallons of effluent daily. This facility has the ability to disinfect less than half of the effluent daily with UV radiation; the remaining effluent is not disinfected and is directed to the injection wells. To protect the public health and designated uses, including recreational use of this popular beach area, the Lahaina WWRF disinfection capacity should be upgraded to treat 100% of the injected wastewater.

The deployment of algal tissue and subsequent nutrient and isotopic analysis to detect nutrient pollution was developed and first used in Moreton Bay in 1997 (Costanzo et al., 2001). After the documentation of clear isotopic signals associated with sewage pollution, the public and governmental agencies expressed discontent in response to the obvious signs of anthropogenic impact in the nitrogen limited western regions of the coastal embayment. This technique has established its value by guiding management decisions in the region and providing essential timely feedback that captured positive outcomes of capital investments, including the results from progressively upgrading the wastewater treatment plants in the area to reduce their N loads into Moreton Bay (Costanzo et al., 2005; Pitt et al., 2009).

Sewage related problems on coral reefs have been documented in Hawai‘i since the 1970’s when Maragos (1972) determined that more than 99% of the coral in the south end of Kaneohe Bay, Oahu died from anoxia and hydrogen sulfide toxicity as a result of treated sewage effluent disposal. Long term impacts of shallow water sewage disposal set off decadal changes in Kaneohe Bay (Smith et al., 1981). Kinsey (1985) later determined that bioeroder communities increased in response to the sewage discharge. Ongoing studies are determining if bioerosion is increased on the reef at Kahekili in comparison to reference sites on the neighboring island of Lanai. In Florida, high human density and associated wastewater loadings were associated with elevated δ^{15}N values in harmful algal blooms where the warm buoyant freshwater effluents discharged from injection wells quickly went to the surface likely affecting shallow mid and deep reefs (Lapointe et al., 2005). In 1996, the State of Florida 120 Administrative Hearing was held to discuss anthropogenic nutrient loading in the Florida Keys, where the hearing officer ruled that land-based nutrients pose a threat to coral reefs and that improved wastewater treatment and nutrient removal must be developed in the Keys. This led to a governmental mandate for the Florida Keys to have central sewage collection and treatment including nutrient removal by July 1, 2010 (Risk et al., 2009).

Injection wells are regulated under the federal SDWA UIC Program which controls the subsurface injection of waste fluids below, into and above underground sources of drinking water. The federal UIC permit conditions, are therefore, concerned with maintaining well integrity and protecting underground sources of drinking water from pollutant levels that would cause exceedances of drinking water quality criteria. The federal UIC program does not require wastewater treatment standards or any requirement related to the impacts of discharges to surface water quality and aquatic resources, including coral reefs. Standards for effluent and ambient water quality are required by the CWA, with the objective “to restore and maintain the physical, chemical, and biological integrity of the Nation’s waters.” The CWA prohibits the discharge of pollutants from point sources into “Waters of the US” except in compliance with a NPDES permit, (nonpoint sources do not require NPDES permits and agricultural runoff is specifically

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exempted from NPDES permitting). CWA Section 401 requires the applicant for a federal permit that would conduct any activity that may result in "any discharges into navigable waters" to obtain a State certification that any discharge allowed by the federal permit meets the technology based effluent standards, water quality standards, water quality based effluent limits, pre-treatment effluent and toxic standards. Section 403 establishes specific guidelines for NPDES discharges into territorial seas and waters of the contiguous zone (oceans), specifying that there be "no unreasonable degradation of the marine environment". NPDES permits implement minimum wastewater treatment standards through the imposition of technology based effluent limits with more restrictive water quality based effluent limits if discharges meeting technology based effluent limits might cause or contribute to exceedances of surface water quality standards. These CWA requirements often result in more restrictive effluent limits (requiring more treatment for pollutant removal) than would be required under a UIC permit. For example, the Maui Ocean Center NPDES discharge is limited to 3.1 kg (7 lbs) d^{-1} of TN, and is subject to further reductions if needed under a TMDL; whereas the County of Maui WWRF UIC permits do not limit the mass discharge of nitrogen and currently inject mass loads estimated to range from 79 to 207 kg (131–457 lbs) d^{-1} of TN. Implementation of pollutant load reduction to meet TMDL allocation is mandatory for discharges authorized under NPDES permits, whereas attainment of allocations for other sources, such as injection wells under federal UIC permits and permit-exempted nonpoint sources, is implemented through voluntary and incentive based programs.

Although injection wells discharge pollutants and are considered point sources under the CWA (40 CFR Part 122.2), NPDES permits have not typically been required because the definition of "Waters of the US" under the CWA does not explicitly include groundwater; jurisdiction has been based largely on the interpretation of the term "navigable waters". Recently a number of courts have held that the NPDES permit requirements of the CWA potentially apply even to the indirect discharge of a pollutant into navigable waters where there is "a connection or link between discharged pollutants and their addition to navigable waters" or significant nexus between source and impact (Rapanos v. US, 547 US 715 (2006); Northern California River Watch v. City of Healdsburg, 457 F.3d 1023, 496 F.3d 993 (9th Cir. 2007); http://www.epa.gov/region/water/groundwater/uic-pdfs/lahaina02/Jeff_SchwartzComments).

6. Implications

This work demonstrates the usefulness of algal δ^{15}N values to distinguish between natural and anthropogenic derived N and to identify the spatial extent of algal blooms that are incorporating anthropogenic derived N sources. The method was identified as an assessment tool with potential for use by the State of Hawai‘i's ongoing Integrated Water Quality Reporting to Congress (SH DOH, 2009). Perhaps more importantly from a management perspective, this work provides a significant nexus between a wastewater source injected into the groundwater and specific surface water quality impacts that prevent the attainment of protected uses such as the conservation of coral reefs and support of aquatic life. Given recent court rulings, the establishment of this connection might lead to a determination that injection wells should be required to have NPDES permits in addition to UIC permits. NPDES permits are mandated to include provisions not required under UIC permits including water quality based limits, and compliance with TMDLs and the ocean discharge criteria under CWA Section 403, whereas the SDWA does not require the consideration of impacts to receiving water uses other than drinking water. Where there is significant nexus to navigable waters, governing authorities should assure that any federal authorization to discharge wastewater, including UIC permits, have a CWA Section 401 certification that the permit conditions are in compliance with the requirements for minimum treatment standards, water quality standards, and water quality based effluent limitations where warranted. With or without an NPDES permit, these releases are a source of nitrogen loading that will be addressed by a TMDL in impaired waters receiving injectate. Releases from injection wells, with or without NPDES permits, cannot lawfully be allowed to cause or contribute to violations of water quality standards, degradation of aquatic ecosystems and non attainment of legally protected beneficial uses.

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