



# Identifying nitrogen sources to thermal tide pools in Kapoho, Hawai'i, U.S.A, using a multi-stable isotope approach



Tracy N. Wiegner<sup>a,\*</sup>, Ambyr U. Mokiao-Lee<sup>b</sup>, Erik E. Johnson<sup>b,1</sup>

<sup>a</sup> Marine Science Department, University of Hawai'i at Hilo, 200 W. Kawili St., Hilo, HI 96720, USA

<sup>b</sup> Tropical Conservation Biology and Environmental Science Graduate Program, University of Hawai'i at Hilo, 200 W. Kawili St., Hilo, HI 96720, USA

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## ABSTRACT

Nitrogen (N) enrichment often results in coastal eutrophication, even in remote areas like Hawai'i. Therefore, determining N sources to coastal waters is important for their management. This study identified N sources to tide pools in Kapoho, Hawai'i, and determined their relative importance using three stable isotopes ( $\delta^{15}\text{N}$ ,  $\delta^{18}\text{O}$ ,  $\delta^{11}\text{B}$ ). Surface waters and macroalgal tissues were collected along 100-m onshore-offshore transects in areas of high groundwater input for three months at low tide. Water samples from possible N sources were also collected. Mixing model output, along with macroalgal  $\delta^{15}\text{N}$  values, indicated that agriculture soil (34%) was the largest anthropogenic N source followed by sewage (27%). These findings suggest that more effective fertilizer application techniques and upgrading sewage treatment systems can minimize N leaching into groundwater. Overall, our multi-stable isotope approach for identifying N sources was successful and may be useful in other coastal waters.

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## 1. Introduction

Worldwide, human activities have increased the delivery of nitrogen (N) to coastal waters often resulting in eutrophication (Nixon, 1995; Smith, 2003). This can lead to phytoplankton and macroalgal blooms and resultant decreases in dissolved oxygen (Nixon, 1995; Smith, 2003; LaPointe et al., 2005; Smith and Smith, 2006). Eutrophication may alter and cause negative effects to coastal, pelagic, and benthic marine communities, food webs, fisheries, and microbial communities (Nixon, 1995; Smith, 2003). One of the main sources of anthropogenic N leading to eutrophication is sewage, which is also a human health hazard in near-shore waters (Boehm et al., 2010). Monitoring and managing the effects of sewage in coastal waters has become a major environmental challenge globally (Prüss-Üstun et al., 2008; Corcoran et al., 2010).

There are several approaches that are used to determine if sewage is present in coastal waters; one of them includes measuring stable isotopes, specifically  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of  $\text{NO}_3^-$  and  $\delta^{11}\text{B}$  in the water column and  $\delta^{15}\text{N}$  in benthic macroalgae and animals tissues (e.g., corals, oysters) (Hunt, 2006; Risk et al., 2009; Dailer et al., 2010). The  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of  $\text{NO}_3^-$  can be used to identify sewage and other  $\text{NO}_3^-$  sources,

assuming each source has a distinct isotopic signature (Chang et al., 2002; Seiler, 2005; Hunt, 2006; Table 1). These isotopes can also be used to determine whether the  $\text{NO}_3^-$  has been transformed by microbial processes (e.g., denitrification) since leaving the source (Hunt and Rosa, 2009).  $\delta^{11}\text{B}$  is used as a co-migrating discriminator of  $\text{NO}_3^-$  sources because it is not affected by microbial transformations like N and O in  $\text{NO}_3^-$ . It has been used to trace sewage pollution in groundwater and surface waters as B is found in fabric whiteners, a common constituent of domestic wastewater (Leenhouts et al., 1998; Seiler, 2005; Hunt, 2006). The  $\delta^{15}\text{N}$  signature of macroalgae tissues is used to determine if macroalgae are taking up sewage N, or N from other sources, and incorporating it into their tissues (Umezawa et al., 2002; Savage and Elmgren, 2004; Lin et al., 2007). Often, opportunistic macroalgal species are used as bioindicators as their growth is stimulated by increased N and their tissues reflect the isotopic composition of the N they consumed (Duarte, 1995; Cohen and Fong, 2006). Macroalgae are favored over phytoplankton as bioindicators in this respect because they minimally discriminate between  $^{14}\text{N}$  and  $^{15}\text{N}$  during uptake compared to phytoplankton (Costanzo et al., 2005; Savage, 2005; Swart et al., 2014). The combined use of  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  in  $\text{NO}_3^-$ ,  $\delta^{11}\text{B}$  dissolved in water, and  $\delta^{15}\text{N}$  in macroalgae tissues allows for identification of anthropogenic N sources to coastal waters because sewage and fertilizers have dissimilar isotopic compositions from one another, and often from natural sources, such as soil and ocean  $\text{NO}_3^-$ , too.

Stable isotope studies identifying N sources to fresh- and coastal waters have primarily been conducted in temperate environments using a two isotope approach (Aravena et al., 1993; Pardo et al., 2004), with a

\* Corresponding author.

E-mail addresses: [wiegner@hawaii.edu](mailto:wiegner@hawaii.edu) (T.N. Wiegner), [ambyrmllee@gmail.com](mailto:ambyrmllee@gmail.com) (A.U. Mokiao-Lee), [erikej@hawaii.edu](mailto:erikej@hawaii.edu) (E.E. Johnson).

<sup>1</sup> Present address: University of Hawai'i at Hilo Analytical Laboratory, 200 W. Kawili St., Hilo, HI, U.S.A. 96720

**Table 1**  
Range of reported stable isotope values (‰) for different environmental sources.

Source	$\delta^{15}\text{N-NO}_3^-$	$\delta^{18}\text{O-NO}_3^-$	$\delta^{11}\text{B}$
Sewage	+7 to +20 <sup>a,b,c,d</sup>	-5 to +11 <sup>a,e,f</sup>	-7 to +32 <sup>h,i</sup>
Fertilizers	-5 to +5 <sup>a,b,c,d</sup>	+17 to +25 <sup>a,b,d</sup>	-8 to +17 <sup>i,j</sup>
Soils	-10 to +15 <sup>a,b,c,d</sup>	-10 to +16 <sup>a,d</sup>	-5 to 0 <sup>k</sup>
Groundwater	-4 to +15 <sup>d</sup>	+1 to +6 <sup>e</sup>	-8 to +60 <sup>k,l</sup>
Seawater	+2 to +7 <sup>c,g</sup>	0 to +5 <sup>g</sup>	+40 <sup>l</sup>
Atmospheric N <sub>2</sub>	0 <sup>a</sup>	-	-
Atmospheric O <sub>2</sub>	-	+23 <sup>a</sup>	-

<sup>a</sup>Kendall, 1998; <sup>b</sup>Hunt, 2006; <sup>c</sup>Derse et al., 2007; <sup>d</sup>Xue et al., 2009; <sup>e</sup>Aravena et al., 1993; <sup>f</sup>Xue et al., 2012; <sup>g</sup>Casciotti et al., 2008; <sup>h</sup>Widory et al., 2005; <sup>i</sup>Vengosh et al., 1994; <sup>j</sup>Seiler, 2005.

few recent ones having used their data in mass-balance mixing models to assign percent contributions to N sources (Deutsch et al., 2006; Voss et al., 2006). Unfortunately, this latter approach does not take into account isotopic fractionation, isotope ratio variability of  $\text{NO}_3^-$ , and often there are too many  $\text{NO}_3^-$  sources to an environment for the mass-balance mixing model to be solved exactly (Xue et al., 2009). It is recommended that three or more stable isotopes be used, as well as a mixing model that can account for these uncertainties, like the Bayesian ones now currently being used for food web analyses (Xue et al., 2009; Parnell et al., 2010). Presently, there are a few studies that have used  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of  $\text{NO}_3^-$  in combination with  $\delta^{11}\text{B}$  to distinguish  $\text{NO}_3^-$  sources in fresh- and coastal waters (Leenhouts et al., 1998; Seiler, 2005; Hunt, 2006; Xue et al., 2014). In the last three years, several studies have used Bayesian isotope mixing models to determine percent contribution of different  $\text{NO}_3^-$  sources with  $\delta^{15}\text{N}$ - and  $\delta^{18}\text{O-NO}_3^-$  data (Xue et al., 2012, 2014; El Gaouzi et al., 2013; Yang et al., 2013; Ding et al., 2014), but to our knowledge, only one study to date has used this approach in coastal waters—temperate coastal waters (Korth et al., 2014). Lastly, no study yet has employed these three stable isotopes with a mixing model and  $\delta^{15}\text{N}$  measurements in macroalgal tissues to trace sewage pollution. Together, these measurements will allow for the dominant  $\text{NO}_3^-$  sources to a water body to be identified with greater confidence, as well as provide a more holistic view of N pollution to and within a system.

There is a great need to use an approach like this in tropical coastal waters, especially those with coral reefs. Most coral reefs are located in developing countries where poorly treated or raw sewage enters the coastal waters (Shahidul Islam and Tanaka, 2004), and sewage is one of the primary threats to coral reefs worldwide (Wear and Vega Thurber, 2015). Even in developed nations where coral reefs are located (i.e., Hawaii, U.S.A.), sewage treatment is inadequate (Whittier and El-Kadi, 2014). With the projected growth in the global population by 2 billion people over the next 35 years (Gerland et al., 2014), and preferential growth in tropical coastal regions (Neumann et al., 2015), the amount of sewage entering nearshore waters will increase in the absence of significant intervention.

An ideal place to use this approach to determine sewage inputs to tropical coastal waters is Hawaii because it has the highest usage of gang and domestic cesspools of any state in the United States (USEPA, 2011), permeable substrate, high rainfall, large amounts of groundwater entering coastal areas, and high recreational use of the ocean (Oki et al., 1999). In particular, Hawaii is a good location for this type of research because it is estimated that 77% of the county's population is serviced by cesspools, which is approximately 50,000 units, comprising 56% of those in the state (Whittier and El-Kadi, 2014). Objectives of this study were to identify the potential sources of N to a tide pool ecosystem located on Hawaii Island, determine their relative percent contributions to tide pool water  $\text{NO}_3^-$  using  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of  $\text{NO}_3^-$  and  $\delta^{11}\text{B}$  in a mixing model, and assess which of these N sources are being used by benthic macroalgae by measuring  $\delta^{15}\text{N}$  in their tissues. This study is the first to use this combination of isotopes together with a mixing model to determine N inputs to tropical coastal waters and

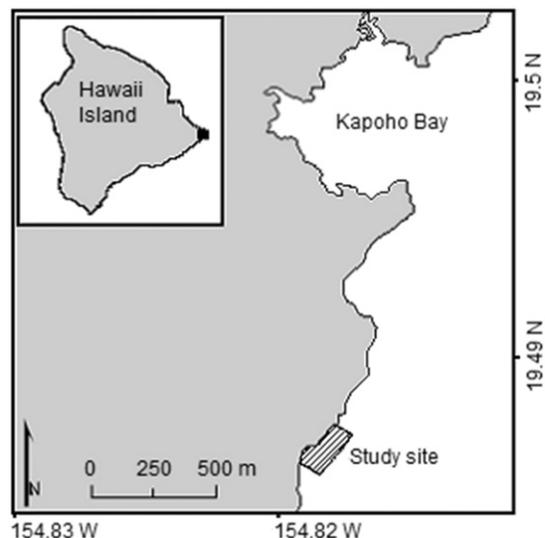
examine their spatial variability within a system. If this approach is successful in identifying N sources to this tide pool system, it could be adopted elsewhere for watershed management purposes.

## 2. Materials and methods

### 2.1. Site description

The study site was the Wai'Ōpae tide pools in Kapoho, located on the east side of Hawaii Island, about 30 km southeast of Hilo, Hawaii (Fig. 1). Kapoho's watershed is ~6 km<sup>2</sup>, with an elevation and population of 183 m and 8,587 people, respectively. Land use is primarily agriculture (80% of total land area), consisting of papaya, orchid, and anthurium farms, with the remainder of the land comprised of forests (8%), barren land (11%), and urban areas (1%). Sewage inputs from cesspools are concerning at the Wai'Ōpae tide pools, where the land is subsiding (0.24 m since 1975) and most homes adjacent to the tide pools are serviced by cesspools and a few by septic systems (Engineering Concepts, Inc., 2010). Hawaii Department of Health (HDOH) records were incomplete about the use of individual wastewater systems (IWS) at Vacationland Estates; however, their data indicate that on-site units consist of cesspools (33%), septic systems (21%), and aerobic treatment units (5%) (Engineering Concepts, Inc., 2010). Because of these conditions, Kapoho was assigned the highest risk score for cesspool contamination of coastal waters in a recent report to Hawaii State (Whittier and El-Kadi, 2014). The area is also designated as a Special Management Area (SMA), a Critical Wastewater Disposal Area (CDWA), and a Marine Life Conservation District (MLCD), so there is a strong desire and mandate to ensure good water quality.

Additionally, the Wai'Ōpae tide pools are located downslope of Hawaii's most active volcano, Kilauea, and their shoreline is relatively unique to the Hawaiian Islands due to the abundance of geothermally-heated groundwaters entering the coast (Juvik and Juvik, 1998). Groundwater is the primary freshwater source entering and transporting N to the tide pools, as there are no perennial streams in the region. The Wai'Ōpae tide pools and their watershed also receive, on average, 200 cm of rain near-shore and 400 cm of rain upslope annually (Juvik and Juvik, 1998). As a result of the conditions at the Wai'Ōpae tide pools, the community and county are concerned that sewage may be transported by groundwater at low tide and impacting the tide pool ecosystem, which supports an abundance of corals and marine organisms and is used recreationally by the local population and tourists.



**Fig. 1.** Location of the Wai'Ōpae tide pools, Kapoho, Hawaii, U.S.A.

## 2.2. Study approach

The goal of this study was to determine the relative percent contributions of different N sources entering the Wai'ōpae tide pools using stable isotope signatures of surface waters and benthic macroalgae. To accomplish this, potential N sources (sewage, agriculture soil, groundwater, ocean water) were sampled within the watershed and analyzed for  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  in  $\text{NO}_3^-$ , and  $\delta^{11}\text{B}$ , and used for identification of N sources to the tide pools' surface waters and benthic macroalgal tissues. Next, groundwater seeps were identified using high-resolution spatial surface water salinity and temperature mapping, and once the areas were identified, onshore-offshore transects were established for surface water and benthic macroalgae sample collection. The relative percent contributions of the different N sources to tide pool waters were determined using a mixing model which utilized the  $\delta^{15}\text{N}$ - and  $\delta^{18}\text{O}$ - $\text{NO}_3^-$  and  $\delta^{11}\text{B}$  isotopic signatures of the tide pool waters and N sources. A mixing model could not be utilized to determine N source contributions to macroalgal tissues because tissues were only analyzed for  $\delta^{15}\text{N}$  and the model requires two or more isotopes for analysis when assessing multiple sources (Parnell et al., 2010). Thus,  $\delta^{15}\text{N}$  of macroalgal tissues were compared to the  $\delta^{15}\text{N}$  of the N sources to determine where they obtained their N from (Derse et al., 2007).

## 2.3. Sample collection

Possible N sources to the Wai'ōpae tide pools are sewage (septic tank sludge,  $n = 8_{\text{N.O.}}, 4_{\text{B}}$ ), agriculture soil (collected from papaya farms,  $n = 7_{\text{N.O.}}, 4_{\text{B}}$ ), groundwater (collected from drinking water wells upland of agriculture lands,  $n = 14_{\text{N.O.}}, 7_{\text{B}}$ ), and ocean water ( $n = 9_{\text{N.O.}}, 3_{\text{B}}$ ). Three sites per source within the Wai'ōpae tide pools' watershed were sampled January, July, August, and September 2010 during dry conditions for dissolved nutrient concentrations and stable isotopic composition; ocean water was not sampled in January 2010 and only three septic systems were sampled due to limited accessibility of private property. Each groundwater and agricultural soil sample was collected several kilometers apart from one another, while ocean water samples were collected from three locations evenly dispersed across the tide pool area. Fertilizers [14–14–14 (N:Phosphorus (P):Potassium (K)), 16–16–16 (N:P:K)] used on papaya farms (obtained from a local supplier) and agricultural soil samples from various locations within the watershed were collected. For each soil sample, 10 g of dried (at 60 °C for 48 h or until constant weight was obtained) soil was suspended in 100 mL of deionized water, shaken overnight, and filtered through a pre-combusted (500 °C for 6 h) GF/F filter (Whatman™) and a sterile 0.22- $\mu\text{m}$  cellulose acetate filter (Whatman™) for nutrient and stable isotope analyses, respectively. The extract was representative of the leachable soil  $\text{NO}_3^-$  after one rain event and this approach has been used in previous studies (Derse et al., 2007).

Groundwater seeps to Wai'ōpae tide pools were identified during the lowest tidal heights of the year by creating high-resolution spatial surface water salinity and temperature maps. Mapping was conducted from April through November 2009 at low tide when groundwater influence was greatest and easiest to detect. High-resolution spatial surface water mapping was conducted with a YSI™ 6600 V2 multi-parameter sonde, attached to a YSI™ 650 MDS data logger, and a Garmin™ E-Trex global positioning system (GPS), which recorded data every three s, resulting in thousands of data points during a survey. The mapping methodology was modified from Madden and Day (1992). First, the outer edge of the sampling area was delineated with the YSI sonde, data logger, and GPS attached to a floatation device, and then they were towed around in a 'zig-zag' fashion within the delineated boundary. Data were then uploaded into a mapping program (Golden Software Surfer 9.0™), overlaid onto an aerial map, interpolated, and used to create salinity and temperature maps (Fig. 2).

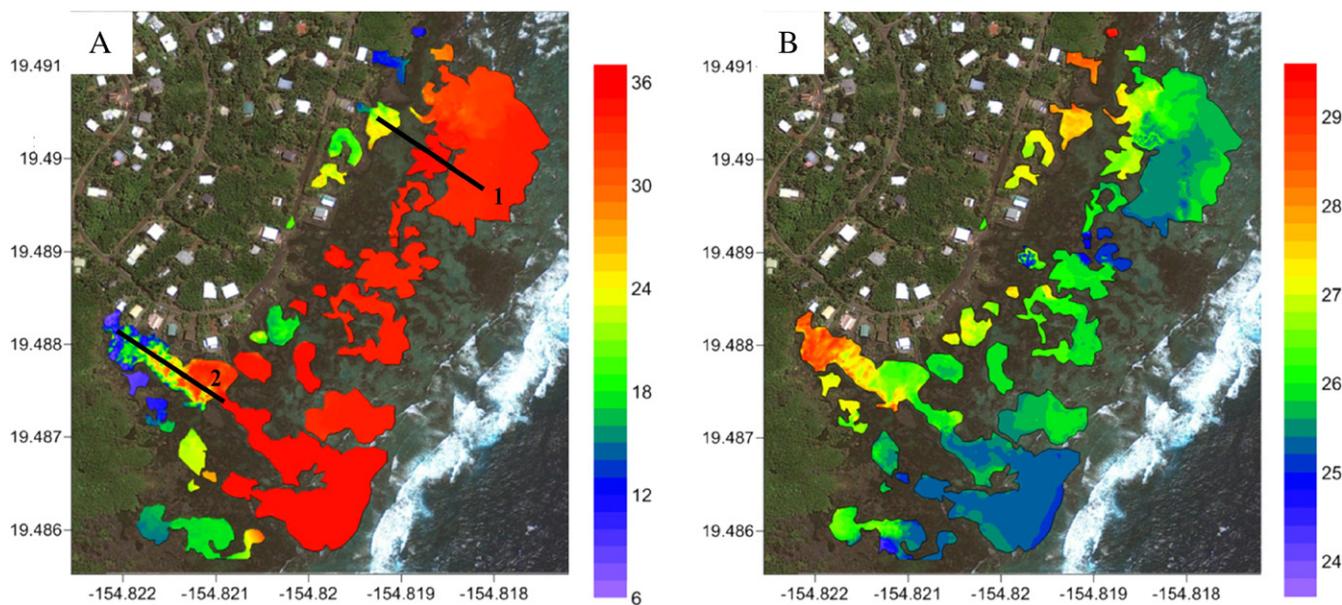
For this study, the two regions with the highest groundwater influence were selected for 100-m onshore-offshore transects for water and macroalgal tissue sample collection. Transect 1 was located outside of the MLCD boundary and transect 2 was located within MLCD boundary (Fig. 2). Tide pool water and macroalgal tissues were collected at low tide, once monthly during July, August, and September 2010. One-liter water samples were collected in acid cleaned, triple sample-rinsed high density polyethylene containers with sample water from the surface (~5 cm depth), where freshwater floats due to density stratification, for a total of eight designated distances along the transect line at 0, 5, 10, 20, 25, 50, 75, and 100 m. These distances were chosen in order to map the spatial extent of sewage N within the tide pools. Water samples for nutrient and isotope analyses were filtered in the field through pre-combusted GF/F and sterile 0.22- $\mu\text{m}$  cellulose acetate filters, respectively, transported on ice to the laboratory, and stored frozen until analysis.

For benthic macroalgae analysis, three replicate tissue samples were collected from the same eight distances (0 through 100 m) along the transect line used for water quality sampling, resulting in 24 samples per transect. Triplicate macroalgal tissue samples were collected to assess the  $\delta^{15}\text{N}$  variability among macroalgal species, distance along transect, and transect locations. Pilot surveys indicated that a variety of algal species were present along the benthic substrate of the tide pools. The macroalgae were identified visually with an Olympus™ CH30 microscope using published identification books (Abbott, 1999; Abbott and Huisman, 2004; Huisman et al., 2007). Macroalgal tissue samples collected in the field were sorted and placed on ice for transport to laboratory, where tissues were dried in an oven at 60 °C to a constant weight, ground, and homogenized using a Wig-L-Bug grinding mill. Approximately, 2 mg of the ground sample were placed in 4 × 6 mm tin capsules for elemental and isotope analyses. Vouchers collected at the time of sampling were preserved in 4% formaldehyde and used for identification purposes. Algal slides were created with 1% aniline blue stain, 1% HCL (used to set stain), and 25% Karo and phenol mixture to seal the cover slip to the slide.

## 2.4. Sample analyses

Nutrients in water samples were measured on a Pulse Technicon™ II autoanalyzer using standard autoanalyzer methods and reference materials (NIST; HACH 307-49, 153-49, 14242-32, 194-49):  $\text{NO}_3^- + \text{NO}_2^-$  [Detection Limit (DL) 0.1  $\mu\text{mol L}^{-1}$ , USEPA 353.4],  $\text{NH}_4^+$  (DL 1.0  $\mu\text{mol L}^{-1}$ , USGS I-2525),  $\text{PO}_4^{3-}$  (DL 0.1  $\mu\text{mol L}^{-1}$ , USEPA 365.5),  $\text{H}_4\text{SiO}_4$  (DL 1.0  $\mu\text{mol L}^{-1}$ , USEPA 366), and total dissolved phosphorus (TDP) (DL 0.5  $\mu\text{mol L}^{-1}$ , USGS I-4650-03). Total dissolved nitrogen (TDN) (DL 5.0  $\mu\text{mol L}^{-1}$ , ASTM D5176) analyses were measured on a Shimadzu™ TOC-V CSH, TNM-1 analyzer and Low Carbon Water and Deep Seawater Reference Material were used (University of Miami, D. Hansell laboratory). Nutrient analyses were conducted at the University of Hawai'i at Hilo Analytical Laboratory.

Analyses of  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  in  $\text{NO}_3^-$  were conducted on a Thermo-Finnigan™ Delta Plus isotope ratio mass spectrometer (IRMS) with data normalized to U.S. Geological Survey (USGS) standards (USGS32, USGS34, USGS35). IAEA-NO3 was used as a check standard. These analyses were conducted at the Northern Arizona University Stable Isotope Laboratory. Analyses of  $\delta^{11}\text{B}$  were conducted on a Finnigan™ Triton multi-collector thermal ionization mass spectrometer with data normalized to NSB951 boric acid. These analyses were conducted at the University of Calgary, Canada, Isotope Science Laboratory. Macroalgal tissues were analyzed on a Thermo-Finnigan™ Delta V Advantage mass spectrometer with a ConFlo III interface and a Costech™ ECS 4010 Elemental Analyzer with data normalized to USGS standard NIST 1547 at the University of Hawai'i at Hilo Analytical Laboratory. Isotopic signatures are expressed as standard ( $\delta$ ) values, in units of parts per mil (‰), and calculated as  $\delta = [(R_{\text{sample}} - R_{\text{standard}}) / R_{\text{standard}}] \times 1000$ , where  $R = {}^{15}\text{N}/{}^{14}\text{N}$ ,  ${}^{18}\text{O}/{}^{16}\text{O}$ , or  ${}^{11}\text{B}/{}^{10}\text{B}$ .



**Fig. 2.** Map of surface water (a) salinity (ppt) and (b) temperature ( $^{\circ}\text{C}$ ) at Wai'ōpae tide pools, Kapoho, Hawai'i, U.S.A. The highest water temperatures were found in conjunction with lowest salinity values, indicating that geothermally heated groundwater was entering the tidal pools. The site was mapped using a YSI 6600 V2 multi-parameter water quality sonde from March to October 2009. Lines and numbers on salinity map indicate transect locations (transects 1 and 2).

## 2.5. Data analyses

The  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  in  $\text{NO}_3^-$ , and  $\delta^{11}\text{B}$  of sources were compared using a one-way analysis of variance (ANOVA). Dissolved nutrient concentrations and  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  in  $\text{NO}_3^-$ , and  $\delta^{11}\text{B}$  of tide pool waters were compared among distances (0, 5, 10, 20, 25, 50, 75, and 100 m) and transect location (transect 1 and transect 2) using a two-way ANOVA.  $\text{NH}_4^+$ ,  $\text{H}_4\text{SiO}_4$ , and  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  in  $\text{NO}_3^-$  data were log-transformed to ensure they met the assumptions of parametric statistics. The  $\delta^{15}\text{N}$  values of macroalgal species that could be identified and the ones which had at least three or more samples collected were compared among species using a one-way ANOVA. Macroalgal tissue  $\delta^{15}\text{N}$  were compared among distances and between transects using a two-way ANOVA. Post-hoc analyses were conducted using the Tukey HSD multiple comparisons test. All statistical analyses were performed in SYSTAT<sup>™</sup> software package v. 11 at  $\alpha$  level of 0.05.

Mixing models in the stable isotope analysis in R (SIAR) v. 4.0 program were used to determine the relative percent contributions of the different N sources to tide pool waters (Parnell et al., 2010; Atwood et al., 2011; Xue et al., 2012). N sources used in this model included sewage, agricultural soil, and groundwater. Both their measured stable isotope values and concentrations were used in the model. Ocean water was not included in the model as originally intended because nitrate concentrations in seven of the nine samples were below detection limits for the stable isotope analysis ( $>2 \mu\text{mol L}^{-1}$ ; Coplen et al., 2012), and therefore, its contribution to the nitrate pool in tide pool water was considered to be negligible. The SIAR mixing model program uses Bayesian methods and is able to reflect natural variation and uncertainty within a system by allowing numerous sources of variability to be incorporated (Parnell et al., 2010). The model estimates the probability distribution for the relative contribution of each source to the mixture (tide pool water) taking into account uncertainty associated with multiple sources and their isotopic compositions. SIAR assumes that the stable isotopic composition of the  $\text{NO}_3^-$  sources are normally distributed, and Ryan Joiner test (similar to Shapiro–Wilk test) was used assess whether this assumption was met with our data.  $\delta^{15}\text{N}$ - and  $\delta^{18}\text{O}$ - $\text{NO}_3^-$  data were normally distributed for all sources, except for three  $\delta^{18}\text{O}$ - $\text{NO}_3^-$  values, one for soil and two for groundwater. Although denitrification was possible in the tide pool waters at some locations on certain dates (dissolved oxygen concentration range 1.80–5.08  $\text{mg L}^{-1}$ , Wiegner unpubl. data),

regression analysis of our  $\delta^{15}\text{N}$ - and  $\delta^{18}\text{O}$ - $\text{NO}_3^-$  data revealed that these isotopes were not being enriched in a 2:1 ratio as expected with denitrification (Kendall, 1998). Hence, a denitrification enrichment factor was not incorporated into SIAR. Lastly, data were analyzed by transect and distance by averaging isotopic values across dates. Due to the few data points from the 75-m distance, this location was excluded as a distance in the mixing model. Percent contributions are reported as the 50% Bayesian credibility interval which allows for a wider range of isotopic variability to be presented (Parnell et al., 2010; Atwood et al., 2011).

## 3. Results

### 3.1. Sources

There were significant differences in nutrient concentrations and isotopic signatures among N sources. Sewage had the highest  $\text{NH}_4^+$ , TDN,  $\text{PO}_4^{3-}$ , TDP, and  $\text{H}_4\text{SiO}_4$  concentrations and the  $\text{NO}_3^-$  was significantly enriched in  $^{15}\text{N}$  and  $^{18}\text{O}$  compared to agriculture soils and groundwater ( $p < 0.0001$  and  $p < 0.0001$ , respectively) (Table 2). Agriculture soil had the highest  $\text{NO}_3^- + \text{NO}_2^-$  and lowest  $\text{H}_4\text{SiO}_4$  concentrations compared to other N sources, while ocean water had the lowest  $\text{NO}_3^- + \text{NO}_2^-$ ,  $\text{NH}_4^+$ , TDN,  $\text{PO}_4^{3-}$ , and TDP concentrations. Ocean water was the most enriched in  $^{11}\text{B}$  compared to all other sources, with groundwater, sewage, and agricultural soil having successively less  $^{11}\text{B}$  (Table 2). The  $\delta^{11}\text{B}$  of groundwater was significantly higher compared to agriculture soil ( $p = 0.033$ ), but similar to sewage (Table 2). While ocean waters were analyzed for stable isotopes of N and O in  $\text{NO}_3^-$ , these data were not statistically analyzed because their values could not be accurately determined due to their nitrate concentrations being below detection limits (Coplen et al., 2012). Fertilizers were also not statistically analyzed in the above ANOVAs because only two samples were taken in this study.

### 3.2. Tide pool water

There were significant differences in dissolved nutrient concentrations from tide pool waters. Dissolved  $\text{NO}_3^- + \text{NO}_2^-$ , TDN, TDP, and  $\text{H}_4\text{SiO}_4$  concentrations were significantly different among distances ( $p = 0.005$ , 0.004, 0.042, and 0.015, respectively); however, only

**Table 2**

Average (S.E.) and range of dissolved nutrient concentrations ( $\mu\text{mol L}^{-1}$ ) and stable isotope signatures (‰) for N sources to Wai'ōpae tide pools, Kapoho, Hawai'i, U.S.A.  $n$  = number of samples; Ag. = Agriculture; bdl = below detection limits (see methods for more details).

Source	$n$	$\text{NO}_3^- + \text{NO}_2^-$	$\text{NH}_4^+$	TDN	$\text{PO}_4^{3-}$	TDP	$\text{H}_4\text{SiO}_4$	$\delta^{15}\text{N}-\text{NO}_3^-$	$\delta^{18}\text{O}-\text{NO}_3^-$	$\delta^{11}\text{B}$
Sewage	8	9.4 (8.3) bdl to 67.6	2456 (502) 1 to 4202	2627 (493) 72 to 4218	176.8 (44.8) 2.2 to 402.0	153.0 (28.3) 2.1 to 230.0	263 (105) 19 to 781	12.30 (1.72) 11.49 to 16.98	16.03 (2.21) 15.51 to 20.00	0.26 (3.28) <sup>a</sup> −8.70 to 7.00
Fertilizers	2	–	–	–	–	–	–	−0.85 (0.55) <sup>b</sup> −1.40 to −0.30	–	–
Ag. Soil	8	64.8 (24.8) 1.5 to 192.0	225 (158) bdl to 1320	584 (245) 23 to 2069	30.9 (16.7) 1.0 to 136.0	43.1 (21.9) 4.5 to 158.0	9 (4) bdl to 31	1.33 (2.16) <sup>c</sup> −4.68 to 9.30	7.06 (1.82) <sup>d</sup> −0.48 to 13.87	−12.08 (5.08) <sup>d</sup> −21.30 to 2.00
Groundwater	20	15.0 (1.1) 8.5 to 27.3	1 (0) bdl to 7	21 (1) 16 to 29	2.7 (0.2) 0.9 to 3.9	3.1 (0.2) 1.2 to 3.9	160 (31) 31 to 436	4.32 (0.68) <sup>e</sup> 1.25 to 7.96	3.57 (0.96) <sup>e</sup> −0.55 to 10.26	10.59 (3.75) <sup>f</sup> −4.70 to 38.00
Ocean	9	0.0 (0.0) bdl to 0.2	0 (0) bdl to 1	7 (1) bdl to 11	bdl	bdl	bdl	bdl	bdl	40.67 (0.64) <sup>g</sup> 39.50 to 41.70

<sup>a</sup>  $n = 4$ .

<sup>b</sup> For  $\delta^{15}\text{N}$  analyses, solid samples of 14–14–14 (N–P–K) and 16–16–16(N–P–K) fertilizers were used.

<sup>c</sup>  $n = 7$ .

<sup>d</sup>  $n = 4$ .

<sup>e</sup>  $n = 14$ .

<sup>f</sup>  $n = 8$ .

<sup>g</sup>  $n = 3$ .

$\text{NO}_3^- + \text{NO}_2^-$  and TDN concentrations significantly differed between transect location ( $p = 0.020$  and  $p = 0.004$ , respectively), with transect 1 having values that were three times higher than transect 2. Dissolved  $\text{NO}_3^- + \text{NO}_2^-$ ,  $\text{NH}_4^+$ , TDN, TDP, and  $\text{H}_4\text{SiO}_4$  concentrations along transect 1 showed a decreasing trend in values from 0 m to 100 m. A similar trend in  $\text{NO}_3^- + \text{NO}_2^-$ , TDN, and TDP along transect 2 was observed; however,  $\text{NH}_4^+$  and  $\text{H}_4\text{SiO}_4$  concentrations showed a slight increase in values from 0 m to 100 m. The  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  in  $\text{NO}_3^-$  and  $\delta^{11}\text{B}$  signatures of tide pool waters were not significantly different among distances or transects sampled. The  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  values of  $\text{NO}_3^-$  in tide pool waters from all distances along both transects have values near agriculture soil and groundwater (Fig. 3). The  $\delta^{11}\text{B}$  signatures of tide pool waters, from all distances, along both transects, have values near ocean water (Tables 2 and 3).

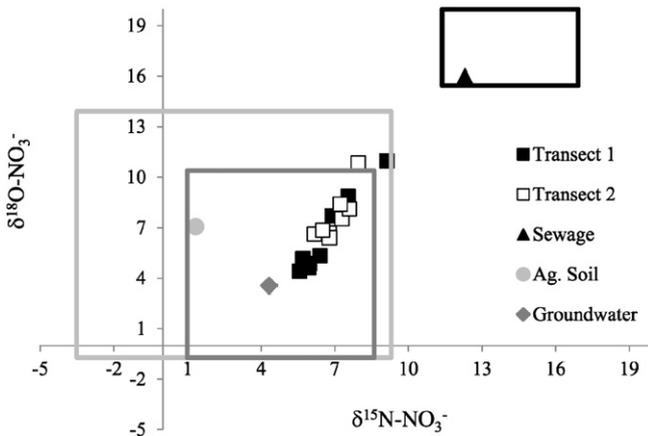
### 3.3. SIAR mixing model

Percent contributions of the N sources were similar when analyzed by transect location in the SIAR mixing model. Therefore, the transect data were combined and re-analyzed for a more robust analysis. The SIAR mixing model was run with and without  $\delta^{11}\text{B}$  data. With the  $\delta^{11}\text{B}$  data, the model identified groundwater (average: 41%, range: 34–43%) as the largest  $\text{NO}_3^-$  source to the tide pool waters among all distances, followed by sewage (36%, 34–42%), and then agricultural soil (23%,

22–24%). Without  $\delta^{11}\text{B}$  data, groundwater (38%, 25–53%) was identified as the largest  $\text{NO}_3^-$  source, followed by agriculture soil (34%, 22–49%), and then sewage (27%, 1–40%) (Table 4).

### 3.4. Macroalgae

The two most common macroalgae found along transect 1 were *Valonia* sp. and *Cladophora* sp. (Fig. 4). The three most abundant macroalgae found along transect 2 were *Amansia glomerata*, *Dichotomaria marginata*, and *Galaxaura rugosa* (Fig. 4). Other macroalgal species found included *Ceramium* (transect 1,  $\delta^{15}\text{N} = 1.70\%$ ,  $n = 1$ ), *Pterocladia* (transect 1,  $\delta^{15}\text{N} = 2.50\%$ ,  $n = 1$ ; transect 2,  $\delta^{15}\text{N} = 1.90\%$ ,  $n = 1$ ), and unknown (transect 1,  $\delta^{15}\text{N}$  (average  $\pm$  S.E.) =  $2.45\% \pm 0.05$ ,  $n = 2$ ). The  $\delta^{15}\text{N}$  of macroalgal tissues significantly differed among species ( $p < 0.0001$ ) (Fig. 4); *Valonia* sp. and *Cladophora* sp. were significantly more enriched in  $^{15}\text{N}$  than *Amansia* sp., *D. marginata*, and *G. rugosa*. Across all species,  $\delta^{15}\text{N}$  ranged between  $-0.90\%$  (*D. rugosa*) to  $4.90\%$  (*Valonia* sp.), with an average of  $1.58\% \pm 1.13$ . Studies that have sampled the  $\delta^{15}\text{N}$  of numerous algal species have analyzed their data by species if all species could be identified and there were replicate samples of each (Cole et al., 2004; Savage, 2005; Dailer et al., 2010). In contrast, studies that compiled  $\delta^{15}\text{N}$  data of different species into a composite value did so because they determined no differences in isotopic values among species and/or the availability of individual species during sample collection were variable (Costanzo et al., 2001; Derse et al., 2007; Dailer et al., 2010). Although macroalgal species from our study had different  $\delta^{15}\text{N}$  tissue values, we grouped all algal species together in order to conduct a two-way ANOVA examining the effect of distance and transect location on  $\delta^{15}\text{N}$  of macroalgal tissues. Algal species were grouped together because all specimens collected could not be positively identified, and common algal species were not found at the two transect locations. The average  $\delta^{15}\text{N}$  of macroalgal species found along transect 1 was significantly higher ( $2.50\% \pm 0.08$ ) than species from transect 2 ( $0.97\% \pm 0.08$ ) ( $p < 0.0001$ , Fig. 5). Within each transect, macroalgal tissue  $\delta^{15}\text{N}$  significantly differed among distances ( $p < 0.0001$ ). For transect 1, macroalgae at the 0-m distance was the most enriched with respect to  $^{15}\text{N}$ . In contrast, macroalgae at the 5-m distance was the most depleted with respect to  $^{15}\text{N}$  along transect 2. When macroalgal tissue  $\delta^{15}\text{N}$  values were plotted by transect against distance from shore and compared with the  $\delta^{15}\text{N}$  values of the N sources from our study, all  $\delta^{15}\text{N}$  of the compiled macroalgal tissues had  $\delta^{15}\text{N}$  values similar to agriculture soil, groundwater, and fertilizers (Fig. 5, Table 2), with most falling within the agriculture soil range.



**Fig. 3.** Biplot of average  $\delta^{15}\text{N}-\text{NO}_3^-$  and  $\delta^{18}\text{O}-\text{NO}_3^-$  (‰) in tide pool waters from Wai'ōpae, Kapoho, Hawai'i, U.S.A. Average stable isotope values for potential N sources to the tide pools, as well as the range in their values (boxes) are shown on figure. Error for average values can be found in Tables 2 and 3.

**Table 3**  
Average (S.E.) salinity (ppt), dissolved nutrient concentrations ( $\mu\text{mol L}^{-1}$ ), as well as  $\delta^{15}\text{N}$ - and  $\delta^{18}\text{O}-\text{NO}_3^-$ , and  $\delta^{11}\text{B}$  values(‰) for Wai'ōpae tide pool waters, Kapoho, Hawai'i, U.S.A. Water samples are reported by transect location (Transects 1 and 2) and distance from shore (0, 5, 10, 20, 25, 50, 75, and 100 m). (\*) indicates where  $\text{PO}_4^{3-}$  concentrations were detectable. bdl = below detection limits (see methods for more details). Sample size ( $n$ ) is three for all transects and distances, except for 75-m distance for both Transect 1 and 2, where  $n = 2$ .

Transect	Distance	Salinity	$\text{NO}_3^- + \text{NO}_2^-$	$\text{NH}_4^+$	TDN	TDP	$\text{H}_4\text{SiO}_4$	$\delta^{15}\text{N}$	$\delta^{18}\text{O}$	$\delta^{11}\text{B}$
1	0	16.50 (1.46)	14.3 (6.2)	4 (2)	25 (5)	0.7 (0.3)*	77 (33)	5.94 (1.14)	4.63 (1.24)	40.05 (0.92)
1	5	16.93 (0.78)	14.4 (1.6)	1 (1)	23 (4)	0.8 (0.3)*	120 (40)	6.91 (2.19)	7.71 (4.80)	39.71 (1.32)
1	10	19.13 (1.73)	11.6 (2.2)	2 (2)	20 (3)	0.7 (0.3)*	89 (22)	5.57 (0.83)	4.41 (1.10)	39.25 (0.81)
1	20	21.27 (0.74)	14.0 (3.0)	1 (1)	21 (1)	0.4 (0.0)*	78 (23)	6.40 (1.14)	5.32 (1.81)	39.47 (0.81)
1	25	23.27 (2.00)	9.5 (1.3)	bdl	18 (1)	0.4 (0.0)	38 (3)	5.96 (1.31)	4.87 (1.70)	40.43 (0.35)
1	50	26.43 (1.47)	9.2 (1.1)	bdl	16 (1)	0.2 (0.0)	57 (13)	5.70 (1.78)	5.16 (1.88)	39.71 (1.32)
1	75	31.00 (0.20)	3.0 (0.3)	bdl	12 (0)	bdl	26 (1)	7.55 (3.19)	8.86 (5.62)	42.73 (0.90)
1	100	33.90 (1.01)	2.1 (1.6)	1 (1)	11 (3)	bdl	24 (11)	9.12 (2.00)	10.97 (2.95)	41.77 (0.85)
2	0	22.17 (0.18)	5.6 (1.0)	bdl	16 (0)	0.8 (0.5)	47 (15)	6.77 (1.14)	6.69 (1.36)	38.80 (2.00)
2	5	18.27 (1.64)	12.0 (3.0)	1 (1)	18 (2)	1.0 (0.7)*	120 (29)	6.78 (1.40)	6.40 (1.27)	38.40 (2.62)
2	10	21.10 (1.24)	9.0 (2.8)	bdl	15 (2)	0.2 (0.1)	102 (35)	7.29 (1.33)	7.54 (1.43)	38.53 (0.79)
2	20	22.30 (1.80)	7.7 (3.2)	bdl	16 (2)	0.3 (0.1)	85 (23)	6.17 (0.86)	6.63 (0.83)	39.57 (0.84)
2	25	22.90 (2.08)	6.7 (2.1)	bdl	14 (2)	0.3 (0.1)	39 (7)	6.51 (1.07)	6.85 (1.28)	39.70 (0.90)
2	50	27.60 (30.10)	3.9 (1.5)	bdl	12 (1)	0.1 (0.1)	48 (19)	7.60 (1.18)	8.11 (2.00)	40.03 (0.78)
2	75	30.40 (6.00)	3.5 (3.5)	bdl	11 (2)	0.1 (0.1)	54 (54)	7.95 (2.24)	10.85 (4.78)	41.91 (0.17)
2	100	28.90 (3.86)	3.1 (2.0)	1 (1)	13 (3)	0.2 (0.1)	50 (26)	7.22 (2.25)	8.40 (3.84)	39.80 (2.34)

## 4. Discussion

### 4.1. N Sources

In regions impacted by N pollution, identifying the dominant sources is important for pollution mitigation and land use management. This can be accomplished, in addition to determining the percent contribution of the different N sources, with stable N isotope measurements and a mixing model. However, to do this successfully, the different N sources must have unique isotopic signatures. The use of two or more stable isotope tracers can help overcome this limitation. In our study, we used stable O and B isotopes in addition to those for N to identify N pollution sources to the Wai'ōpae tide pools, and each N source examined had significantly different  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  in  $\text{NO}_3^-$ , and  $\delta^{11}\text{B}$ . Our  $\delta^{15}\text{N}$  in  $\text{NO}_3^-$  for sewage, agricultural soil, and groundwater fall within the range reported in the literature (Tables 1 and 2). With regards to the  $\delta^{15}\text{N}-\text{NO}_3^-$  values in the agriculture soil we sampled, they had the widest range and this result could be due to timing of fertilizer applications and the fact that soil samples were collected from different farms. We also analyzed  $\delta^{15}\text{N}$  from four fertilizers used in the Kapoho region; however, only two of them (14–14–14 and 16–16–16) had measurable  $\text{NO}_3^-$  concentrations, and their  $\delta^{15}\text{N}$  values were depleted (0.0‰ and –1.3‰, respectively) compared to those in the agriculture soil. The  $\delta^{18}\text{O}$  in  $\text{NO}_3^-$  values of agriculture soil and groundwater from our study fell within the range of literature values (Tables 1 and 2), while the sewage value was more enriched in  $^{18}\text{O}$  compared to literature values (Tables 1 and 2). The  $\delta^{11}\text{B}$  values of sewage, groundwater, and ocean water that were measured in our study fell within the range of literature values (Table 1), while agriculture soil exhibited highly depleted values which could be due to the source of B in the various fertilizers applied on upland papaya farms (Wieser et al., 2001). The  $\delta^{11}\text{B}$  values from

**Table 4**

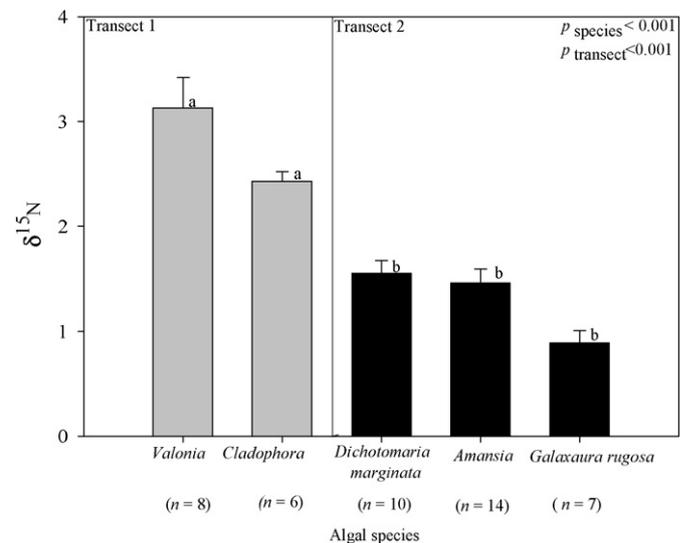
Average (range) relative percent contributions of N sources [sewage, agriculture soil (Ag. Soil), and groundwater] to Wai'ōpae tide pool waters, Kapoho, Hawai'i, U.S.A. from 0, 5, 10, 20, 25, 50 and 100 m from shore. Percent contributions were determined using SIAR v 4.0 and values are reported as the 50% credibility interval. Data from distances along transects were combined for this analysis.

Distance (m)	Sewage	Ag. Soil	Groundwater
0	26 (14–38)	35 (24–48)	40 (26–53)
5	27 (3–34)	35 (24–49)	39 (27–52)
10	26 (1–41)	34 (23–49)	39 (26–52)
20	26 (2–38)	35 (23–49)	40 (27–53)
25	27 (2–40)	35 (23–49)	39 (25–51)
50	28 (4–38)	34 (23–48)	38 (25–51)
100	33 (16–40)	33 (22–47)	34 (25–49)

four fertilizers (ammonium nitrate, urea, and phosphate-based products) ranged from –2‰ to 14.8‰, and are attributed to the B mineral used in fertilizer production (Komor, 1997; Wieser et al., 2001). Foliar applications of borax and ground applications of elemental B fertilizers are recommended for papayas to prevent and reduce B deficiencies that cause bumpiness or deformities in the fruit. Due to a large range in isotopic compositions of B in the literature that are derived from various B sources, further investigations of  $\delta^{11}\text{B}$  for B sources are needed to better utilize  $\delta^{11}\text{B}$  values to trace pollution and determine their relative contributions.

### 4.2. Tide pool water

$\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  in  $\text{NO}_3^-$  and  $\delta^{11}\text{B}$  along upland-lowland gradients have been used to determine N sources to groundwater in several studies (Leenhouts et al., 1998; Seiler, 2005; Hunt, 2006, 2014; Xue et al., 2014). However, to our knowledge, our study is the first to apply this multi-isotope approach to detect N sources to coastal waters along an onshore-offshore gradient. Dissolved nutrient concentrations (except



**Fig. 4.** Average (S.E.)  $\delta^{15}\text{N}$  (‰) of macroalgal species from Wai'ōpae tide pools, Kapoho, Hawai'i, U.S.A. Results from a one-way ANOVA are shown on figure ( $\alpha = 0.05$ ). Differences among macroalgal species were examined with a Tukey's test and bars with different letters are significantly different from one another. Other macroalgal species found include *Ceramium* (transect 1,  $\delta^{15}\text{N} = 1.70\text{‰}$ ,  $n = 1$ ), *Pterocladia* (transect 1,  $\delta^{15}\text{N} = 2.50\text{‰}$ ,  $n = 1$ ; transect 2,  $\delta^{15}\text{N} = 1.90\text{‰}$ ,  $n = 1$ ), and unknown (transect 1,  $\delta^{15}\text{N} = 2.45\text{‰} \pm 0.05$ ,  $n = 2$ ).

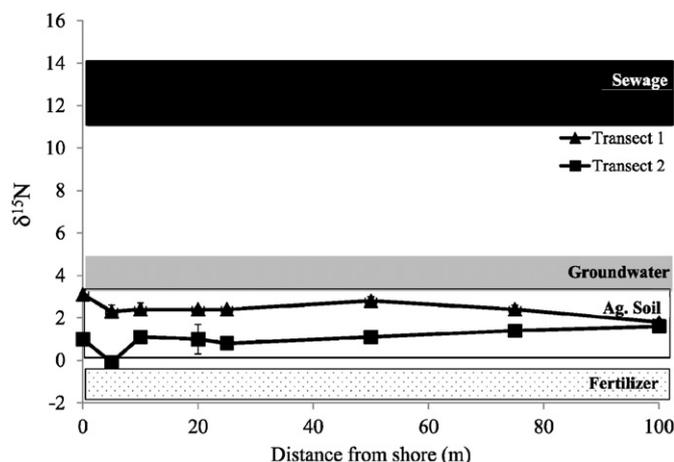


Fig. 5. Average (S.E.)  $\delta^{15}\text{N}$ (‰) of macroalgal tissues found along transects 1 and 2, from Wai'Ōpae tide pools, Kapoho, Hawai'i, U.S.A., with respect to  $\delta^{15}\text{N}$  of the N sources average (S.E.) from this study. Transect 1 is denoted by squares and transect 2 by triangles.

$\text{NH}_4^+$ ) and isotopic compositions of tide pool waters did not significantly vary between transect location, but did vary with distance from shore suggesting that groundwater discharging along the shoreline was the primary source of these constituents; other studies have observed similar patterns (Hunt and Rosa, 2009; Bruland and Mackenzie, 2010). Isotopic signatures of tide pool waters were similar among distances and between transect location suggesting that there was minimal microbial N transformation affecting the  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  in  $\text{NO}_3^-$  with distance from shore. The  $\delta^{15}\text{N}$  in  $\text{NO}_3^-$  values from surface waters were similar in isotopic composition to agriculture soil and groundwater (Fig. 3), suggesting that agriculture soil from the watershed is the main anthropogenic N source to tide pool waters. Dissolved  $\text{NO}_3^- + \text{NO}_2^-$  concentrations decreased with distance from shore suggesting either uptake by phytoplankton and/or denitrification; however, it appears that this latter process was not occurring in the tide pool waters as the  $\delta^{18}\text{O}$  in  $\text{NO}_3^-$  did not significantly vary within the tide pools with increasing distance from shore, and denitrification leaves the residual  $\text{NO}_3^-$  isotopically enriched in  $^{18}\text{O}$  (Aravena et al., 1993; Hunt, 2006; Hunt and Rosa, 2009). The  $\delta^{11}\text{B}$  values were similar among distances and between transect locations, indicative of a well-mixed system, and were close in value to the ocean water end member, suggesting that ocean water was the largest B source to tide pools.

Traditionally, mixing models have been used to assess the percent contributions of food sources to the diets of organisms within food webs (Phillips and Gregg, 2003; Parnell et al., 2010; Atwood et al., 2011). Here, we used mixing models to determine the percent contribution of N sources to a coastal water body along an onshore-offshore transect using  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  in  $\text{NO}_3^-$  and  $\delta^{11}\text{B}$ . While this approach has been used with  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  in  $\text{NO}_3^-$  in several freshwater studies (Xue et al., 2012, 2014; El Gaouzi et al., 2013; Yang et al., 2013; Ding et al., 2014) and a single coastal water one (Korth et al., 2014), no study to date has used it with  $\delta^{11}\text{B}$ . When comparing the SIAR results with and without  $\delta^{11}\text{B}$  data to  $\delta^{15}\text{N}$  values of the macroalgae (Fig. 5), it appeared that the model overestimated the contribution of the sewage to the tide pool  $\text{NO}_3^-$ , as none of our macroalgal samples had  $\delta^{15}\text{N}$  values within the range of sewage (Tables 1 and 2). A bi-plot of  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of  $\text{NO}_3^-$  also shows that the tide pool waters fall within the range of groundwater and agricultural soils, not sewage (Fig. 3). A recent study reported that B isotopes did not work well as a tracer for sewage in brackish waters (Hunt, 2014). They found that in water samples containing 15–20% saltwater, the B pool was dominated by seawater, masking contributions from sources with lower B concentrations (Hunt, 2014). Seawater has an approximate B concentration of  $398 \mu\text{mol L}^{-1}$ , while municipal sewage has a concentration ranging

from 23 to  $93 \mu\text{mol L}^{-1}$  (Leenhouts et al., 1998). Hence, from this point forward, we will be discussing the SIAR results without  $\delta^{11}\text{B}$  data.

We found that agriculture groundwater, soil, and sewage source contributions to tide pool waters were 38%, 34%, and 27%, respectively. The combined percent contribution of anthropogenic N to tide pool waters from agriculture soil and sewage was 61%, while N from groundwater was 38%. High  $\text{NO}_3^- + \text{NO}_2^-$  concentrations near-shore in low salinity waters further suggest that agriculture soil was the main nutrient source to tide pool waters, as  $\text{NO}_3^-$  is often associated with agricultural activities and its concentration was highest in our agricultural soil source (Addiscott et al., 1991; Bruland and Mackenzie, 2010; Bishop et al., 2015). Output from the mixing model is important for land managers, and ours suggest that more effective fertilizer usage and timing of applications will help minimize leaching into the groundwater and improve water quality. Additionally, high  $\text{NH}_4^+$  concentrations at 0 m on transect 1 suggest that sewage may be entering the tide pools at this location as there was a higher concentration of homes adjacent to this transect. While sewage contributed a smaller percentage of the total N compared to agriculture soil, the residential area contributes two orders of magnitude more N than agriculture fields on an aerial basis. It is projected that population growth and nutrient loads will increase in the future; these factors could have profound impacts on the tide pool ecosystem by potentially altering the N sources and forms, and increasing the quantity. These changes in the N flux could stimulate excess macroalgal growth, possibly resulting in a phase-shift from a coral- to an algae-dominated ecosystem, like observed on Caribbean, Indonesian, and Australian reefs (Hughes, 1994; McManus and Polsenberg, 2004). Increases in agriculture could also contribute to these types of conditions, and therefore, it is important for land managers to have information on percent N source contribution, like those from the mixing model, to make informed decisions.

#### 4.3. Macroalgae

Worldwide,  $\delta^{15}\text{N}$  values in macroalgal tissues have been shown to be effective bioindicators of N pollution in water bodies (Cohen and Fong, 2006; Savage, 2005; Smith et al., 2005; Risk et al., 2009; Dailer et al., 2010). In this study, there were significant differences in  $\delta^{15}\text{N}$  tissue values among macroalgal species. Differences in  $\delta^{15}\text{N}$  of macroalgal tissues may be due to light conditions, nutrient availability, and plant physiology (Cole et al., 2004; Smith and Smith, 2006; Bannon and Roman, 2008; Carballeira et al., 2014; Swart et al., 2014). Water depth along transects did not exceed ~2 m which allowed for ample light to reach the benthic macroalgae and light levels were consistent among distances and transects. Water column stratification was also assessed, and there was no horizontal (8.16‰ at 0-m, 8.73‰ at 100-m) or vertical (8.52‰ surface, 8.36‰ bottom) differences in  $\delta^{15}\text{N}-\text{NO}_3^-$ . Also, nutrient concentrations and isotopic signatures of surface waters were similar between transect location. All three of these findings suggest that light and nutrient availability were not factors contributing to significant differences in the  $\delta^{15}\text{N}$  among macroalgal species, but that differences in plant physiology played a role.

There are many physiological attributes that could lead to differences in isotopic values of algal tissues, including: nutrient uptake, growth rates, part of the plant sampled, age of plant tissue, phylum, taxonomic and functional-form groups, and successional stages (Lobban and Harrison, 1994; Carballeira et al., 2014). The algal species found along transect 1 consisted of green and red algae, while only red algae was found along transect 2 (Fig. 4); however, most algal species in our study are late-successional species which are not typically used as bioindicator species in isotope studies (Umezawa et al., 2002; Cohen and Fong, 2006; Dailer et al., 2010). Early-successional opportunistic algae, such as *Ulva* sp., *Gracilaria* sp., and *Cladophora* sp., are typically used in these studies because of their rapid growth rates in response to increased nutrients (Ryther et al., 1981; Peckol and Rivers, 1995; Cohen and Fong, 2006); only one of these species was found

(*Cladophora* sp.) in our study, and only on one transect. However, late-successional algal species are able to incorporate a larger record of nutrient sources over time and should be considered as potential study species for future isotope studies instead of early-successional ones. The macroalgal genera reported in this study exhibit varying physiological characteristics in their phylum and successional stages, which play vital roles in nutrient uptake and N storage (Lobban and Harrison, 1994). These factors may be responsible for the significant differences in the  $\delta^{15}\text{N}$  of the algal tissues; however, without further testing, the exact physiological characteristics that affect the  $\delta^{15}\text{N}$  of macroalgal tissues from Wai'Ōpae tide pools cannot be conclusively determined.

Many studies have effectively used  $\delta^{15}\text{N}$  in macroalgal tissues to trace N pollution because their tissues reflect the source and availability of nutrients over time (Umezawa et al., 2002; Smith et al., 2005; Derse et al., 2007; Dailer et al., 2010). The  $\delta^{15}\text{N}$  of macroalgal tissues in this study fell within the range reported for soil  $\text{NO}_3^-$  impacted by fertilizers (Table 1), with an average  $\delta^{15}\text{N}$  across all species of  $1.58\text{‰} \pm 1.13$  (Fig. 4), and no offshore gradient in signatures was observed. Surface waters collected from the same distances along both transects that macroalgal tissues were collected also suggest that agriculture soils are the dominant anthropogenic N source to tide pool waters. Similar work utilizing  $\delta^{15}\text{N}$  in macroalgal tissues as bioindicators of N source pollution typically report enriched  $\delta^{15}\text{N}$  values in tissues which are attributed to sewage N pollution (Umezawa et al., 2002; Costanzo et al., 2005; Bannon and Roman, 2008; Dailer et al., 2010). Results from studies implicating sewage as the dominant N source to macroalgal tissues have been in areas primarily serviced by sewage treatment facilities with either injection wells or outfalls, and not necessarily from on-site units such as cesspools or septic systems. On-site treatment units, like those at Wai'Ōpae, are spatially diffuse compared to injection wells and sewage outfalls and this may lead to their inputs not being detected or their contributions to the N pool being small compared to other sources. A recent study conducted on Maui found this to be the case where  $\text{NO}_3^-$  from agriculture overwhelmed smaller contributions from cesspools and septic tanks (Bishop et al., 2015).

## 5. Conclusion

N delivery to coastal waters is increasing worldwide with the growing human population and expanding urbanization, industrialization, and agriculture, and the result is widespread eutrophication (Nixon, 1995). Eutrophication has led to phytoplankton and macroalgal blooms, decreases in dissolved oxygen, changes in pelagic and benthic communities and habitats, food webs alterations, and collapse of fisheries in many places (Nixon, 1995). Therefore, knowing where the N is coming from within watersheds is paramount for better management and sustainability of our coastal waters. We found that stable isotopes of N and O in surface water  $\text{NO}_3^-$  and macroalgal tissues, combined with the use of a stable isotope mixing model was successful in determining the N sources to surface waters and benthic macroalgal tissues at the Wai'Ōpae tide pools. Mixing model results served as an important tool in determining the relative percent N source contributions to the tide pool  $\text{NO}_3^-$  and showed that agriculture soil was the dominant anthropogenic N source to tide pool waters and benthic macroalgal tissues. This approach may be useful for other coastal areas experiencing increased N loading, especially in areas where knowledge is lacking on the source(s) of N within the watershed. With this type of information, communities, land managers, and policy makers can make informed decisions regarding land-use that may help improve coastal water quality and allow coastal systems to recover from nutrient pollution.

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