## Tracking Arroyo Colorado Nitrogen into the Lower Laguna Madre

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#### Introduction

Impacts from the Arroyo Colorado (Arroyo), the major gaged source of Lower Laguna Madre (LLM) freshwater are a major concern as a factor causing some of the observed seagrass changes (change in species and spatial distribution). For the most part, this concern is directed towards nutrient loading, due to the Arroyo's role in draining wastewater and agricultural return flows from the LRGV to the LLM (TCEQ, 2006). The Arroyo has been the target of TMDL assessment and regulatory action for the past decade (TCEQ, 2006) for oxygen depletion in part due to high algal densities fueled by high nutrient levels. Ambient nutrient levels in the Arroyo are higher than in the Laguna Madre and nutrient loading increases with higher inflow conditions. Recent monitoring surveys of salinity and nutrient loading parameters have been undertaken, in combination with changes in seagrass biomass and macroalgae (DeYoe unpublished data).

Monitoring seagrasses as indicators of water quality degradation has the main objective of detecting sub-lethal seagrass impacts prior to bed-scale, physical loss of seagrass cover. In the case of LLM seagrass, nutrient additions (nitrogen and phosphorus) could have positive and negative, as well as direct and indirect impacts, on seagrass. Lee (1998) showed that Thalassia at one LLM site responded positively to ammonia additions with increased growth. In an example of a direct negative effect, Burkholder et al. (1994) found that nitrate in excessive amounts had a detrimental effect on the growth of Z. marina, a temperate zone species, due to its physiological characteristics; while the same nitrate treatment produced modest to substantial growth increase in the seagrasses, *Halodule* and *Ruppia*, respectively. However, *H. wrightii* is inhibited at high nitrate levels (100 µM) while Ruppia maritima is inhibited by high ammonia levels (Burkholder et al., 1994). Long-term experimental fertilization of a Thalassia testudinum bed eventually led to its replacement by Halodule wrightii (Fourgurean et al., 1995). Direct responses by Thalassia and Syringodium to nitrogen loading have not been well-characterized. Indirect effects of nutrient enrichment include stimulation of the growth of phytoplankton, macroalgae and seagrass epiphytes that can lead to reduced seagrass productivity due to light reduction (McGlathery 1995). Nutrient addition alone can lead to positive or negative effects on seagrasses, with negative effects typically occurring at higher loading rates. In the case of excessive macroalgae accumulations, shading occurs from macroalgae overgrowing and smothering the seagrasses.

If nutrients (N or P) are limiting, there may be an increase in seagrass production with increases in nutrients or the relative abundance of seagrass species may change. Alternatively, added nutrients may decrease underwater light for seagrass due to reduction in water clarity from enhanced phytoplankton growth or by encouraging the growth of epiphytes on seagrass leaves. In addition, added nutrients may lead to growth of macroalgae (seaweed) resulting in drifting macroalgal mats that can smother seagrasses. Thus, it is considered important to determine the nutrient loading potential for the Arroyo Colorado inflow plumes under a range of flows, from low to high, and during different seasons.

Based on water quality data from TCEQ and flow data from the USGS for the Harlingen gage, average seasonal daily nutrient loading rates were calculated (Table 1). Average annual nutrient loading rates from a 1995 TGLO Arroyo study were also obtained for a tidal site near the confluence of Arroyo with the LLM (TGLO 1995): DIN (dissolved inorganic nitrogen) was 1117

kg N/day and total phosphate was 450 kg P/day. Even though different data sets were used, the nutrient loading rates estimated by these two studies are similar. The Arroyo contributes significant amounts of nitrogen and phosphorus to the LLM and nutrient loading rates tend to be higher in winter (Dec-Feb) and spring (Mar-Jun) than summer (Jul-Sep) and fall (Oct-Nov). This occurs despite the fact that high flow events in the Arroyo are more likely to occur in the summer and early fall. Besides flow, other factors could affect nutrient loading rates such as temperature (water and air) and agricultural fertilization and irrigation activity.

Table 1. Average seasonal daily loading rates of dissolved inorganic nitrogen (DIN) and total phosphate (TP) at the Port of Harlingen based on TCEQ water quality data from 1978 to 2009 and IBWC gage data at Port of Harlingen.

	Flow 5-day			Avg	SD	Avg	SD	Avg
	avg	DIN	ТР	DIN Load	DIN Load	P Load	P Load	Load N/P ratio
	acre-ft/day	n	n	kg/day	kg/day	kg/day	kg/day	(molar)
Winter	427.5	38	11	1380	1962	496	347	6.4
Spring	569.4	46	7	1319	1579	924	1094	3.3
Summer	446.8	46	10	990	1935	344	78	6.6
Fall	548.3	31	8	957	1045	716	736	3.1

There are various possible fates of Arroyo nutrients (N and P) once they enter the LLM. Nutrients could be taken up by bacterioplankton, phytoplankton, benthic microalgae, macroalgae, seagrass epiphytes and/or seagrasses. In addition, LLM sediment can absorb and retain nutrients, nutrients can pass out of the system to the Gulf of Mexico or nitrogen can be lost to the atmosphere through denitrification. As mentioned earlier, stimulation of the growth of phytoplankton, seagrass epiphytes and/or drifting macroalgae can negatively impact seagrass by depriving them of light so it is important to know where nutrients go once they enter the LLM. Nitrogen is of special interest in marine ecosystems as nitrogen is typically found to be limiting. In the LLM, nitrogen was found to be limiting for turtle grass, *Thalassia testudinum* (Lee 1998; Lee and Dunton, 2000).

Nitrogen isotopes have been used as a tool over the past 30 years to track the fate of nitrogen in aquatic systems (Costanzo et al., 2001; Oczkowski et al. 2008). In this method, two stable isotopes of nitrogen, <sup>14</sup>N and <sup>15</sup>N with the former being more abundant, are used to identify sources of nitrogen because the ratio of <sup>15</sup>N to <sup>14</sup>N is distinctive for different sources (Fig. 1). For example, sewage nitrogen is enriched in <sup>15</sup>N while fertilizer nitrogen is not. In practice, the ratio of <sup>15</sup>N/<sup>14</sup>N in the material is compared to a world-wide standard and the relative amount of <sup>15</sup>N or  $\delta^{15}$ N is calculated as follows:

 $\delta^{15}N(0/00) = (Rsample/Rstandard-1) \times 10^3$ 



Fig. 1 . The range in  $\delta^{15}$ N values for a variety of nitrate sources and sinks. Box plots illustrate the 25th, 50th and 75<sup>th</sup> percentiles; whiskers indicate the 10th and 90th percentiles; circles are outliers. From Xue et al. (2009).

To assess the fate of Arroyo nitrogen in the LLM, we collected different kinds of primary producers at varying distances from the Arroyo Colorado and analyzed them for their isotopic nitrogen content. It is expected that the high  $\delta^{15}$ N values of the Arroyo due to sewage input would diminish as one moves away from the Arroyo and that the decreasing trend should be more abrupt going south from the Arroyo due to the general movement of Arroyo water northward caused by prevailing southeasterly winds.

**Methods:** On August 18, 2011, 27 sites arrayed along a N-S transect and an E-W transect (Fig. 2) were visited for collection of seagrass (*Halodule wrightii*), macroalgae (mostly *Palisada poiteaui*), and seagrass epiphytes. The tissue samples were rinsed, cleaned, dried, ground and then analyzed for C and N content and stable C and N isotope ratios by the University of Alaska Fairbanks Stable Isotope Laboratory. Total phosphorus (TP) content of tissue was measured by the method of Solorzano and Sharp (1980) at the UTPA DeYoe lab. In addition to the August 2011 samples, archived samples of seagrass and algae collected during other studies were also analyzed as above.



Fig. 2. Green dots represent the sites in the Lower Laguna Madre visited on August 18, 2011 for collection of seagrass, drift algae and seagrass epiphytes analyzed for N isotopes. This isotope sampling design was laid out according to 2008 salinity plumes (red polygons in figure) observed as output from TXBLEND hydrodynamic modeling.

### **Results and Discussion**

 $\delta^{15}$ N values of primary producers were expected to be high in and near the Arroyo Colorado due to a significant amount of the Arroyo nitrogen being derived from wastewater treatment plants having a high  $\delta^{15}$ N value. Periphyton (attached microalgae) collected from the Arroyo, as expected, had very high  $\delta^{15}$ N values (Table 2).

Table 2.  $\delta^{15}$ N values of periphyton collected from two sites in the tidal segment of the Arroyo Colorado. River Ranch is nearer Rio Hondo while Thomae Park is downstream and nearer the confluence of the Arroyo and LLM.

Collection			d <sup>15</sup> N
Date	Site	Туре	(o/oo)
4/22/2011	River Ranch	periphyton	10.48
4/22/2011	Thomae Park	periphyton	12.47
8/2/2011	River Ranch	periphyton	16.9
8/2/2011	Thomae Park	periphyton	16.87

In the LLM, along the N-S transect  $\delta^{15}$ N values were lower than Arroyo periphyton values and there was no discernible spatial trend in seagrass  $\delta^{15}$ N values which ranged from 2 to 7 (Fig. 3). Data for the seaweed, *Palisada poiteauii* and the *Halodule* epiphytes (not shown) also did not show a trend along the N-S transect and did not match the N-S pattern seen for *Halodule*. All three data sets had low  $\delta^{15}$ N values at the northernmost site near the Port Mansfield Pass suggesting little Arroyo influence in this area of the LLM. Part of the reason that the August 2011 N-S transect may lack a clear trend is that the North Floodway can carry runoff and treated effluent to the LLM like the Arroyo but enters the LLM about 7.5 km (4.6 mi) north of the Arroyo so the influence of the inflow is spread over a wider area becoming more diffuse. In fact, there is a subtle increase in  $\delta^{15}$ N values from 10 to 15 km north of the Arroyo and the North Floodway northward in the LLM which would spread inflow from the Arroyo and the North Floodway northward.

In contrast, the isotopic signatures along the E-W transect did show a distinct decreasing trend from west to east for the seagrass and a discernible but less pronounced trend for the seaweed and the seagrass epiphytes (Fig. 4).

The archived seagrass samples collected along a N-S transect show a trend of increasing  $\delta^{15}$ N values closer to the Arroyo (Fig. 5). These transects had fewer sites but extended about 30 km further south than the August 2011 transect data which may explain why a more noticeable trend is seen. The 2007-08 *Halodule* data also indicates that the nitrogen isotopic signature of the Arroyo can be seen during most seasons of the year.

As would be expected, a dilution effect of the Arroyo nitrogen is seen as  $\delta^{15}$ N values of the LLM primary producers are lower than those for the periphyton that was collected in the Arroyo Colorado. The east-west transect data (Fig. 4) and the archived sample data (Fig. 5) indicates that nitrogen from the Arroyo is being utilized by primary producers (seagrass, seaweed and seagrass)

epiphytes) in the LLM. The 2011 N-S transect data lacks a clear trend most likely due to the diffuse distribution of Arroyo nitrogen along the N-S transect.



Fig. 3.  $\delta^{15}$ N values for the seagrass *Halodule wrightii* collected from the LLM along the northsouth transect at varying distances from the Arroyo Colorado (at 0 km) on 18 August 2011. Negative values are south of the Arroyo while positive numbers are north of the Arroyo.



Fig. 4.  $\delta^{15}$ N values of the seagrass *Halodule wrightii* (top), the macroalgae *Palisada poiteauii* (middle) and epiphytes from *H. wrightii* (bottom) collected on August 18, 2011 in the LLM along the west to east transect starting near the Arroyo Colorado confluence.



Fig. 5.  $\delta^{15}$ N values of the seaweed *Palisada poiteauii* (top) and the seagrass *Halodule wrightii* (bottom) collected from the LLM along a north-south transect in 2004 and 2007-08, respectively. Negative values along the X-axis indicate sites south of the Arroyo while positive numbers indicate sites north of the Arroyo Colorado.

We have shown that significant amounts of Arroyo nutrients enter the LLM where they are utilized at least locally by several kinds of primary producers including seagrass epiphytes, drift algae and seagrass and likely by plankton (phytoplankton and bacterioplankton). Plankton and epiphyte growth stimulated by nutrient additions can reduce available light for seagrass thereby lowering seagrass production rates. Drift algae growth if excessive can produce thick mats overtopping seagrass. If a drift algae mat stays in one place too long it can lead to seagrass loss due to light deprivation (Peckol and Rivers 1996) and/or toxic sediment hydrogen sulfide effects (Holmer and Bondgaard 2001). Unfortunately with the data available, we cannot identify critical nutrient loading rates that produce levels of algae harmful (due to light limitation) to seagrass.

The timing of nutrient additions to the LLM is important as additions during cooler months will not likely have as much of an effect as additions during warmer months because

algal and plant growth rates in winter are depressed as they are more a function of the cooler temperatures than nutrient levels. N loading rates are about 25% higher during winter and spring compared to summer and fall (Table 1). This regime would allow seagrass and drift algae to take up and store nitrogen (due to their large size) during cooler months with less competition from plankton and epiphytes. Plankton and epiphytes would likely compete more effectively for summer and early fall nutrient additions due to their small size and potential for rapid growth. The point is, nutrient additions would likely have a more detrimental effect on seagrass during summer and fall than winter or spring due to the greater potential for light reductions caused by rapid growth of plankton and epiphytes. As noted above, drift algae can also have detrimental effects on seagrass but their ability to respond to nutrient additions is slower than the plankton and epiphytes due to their slower growth rates.

Large precipitation events like tropical storms create a more complicated scenario as they can bring in large quantities of freshwater as well as extra nutrients during warmer months. Lowered salinity will, in general, depress metabolic activity of primary producers but the effect is a function of the duration, rapidity and magnitude of the salinity drop as well as the acclimation ability of the organism. Also the severity of the impact can vary with different seagrass species as each has its own salinity tolerance range. If the salinity drop is severe as with Hurricane Alex in 2010, seagrass will then die, which can alter the entire ecosystem. The impact of the 2010 hurricane on the LLM has not been well-studied.

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